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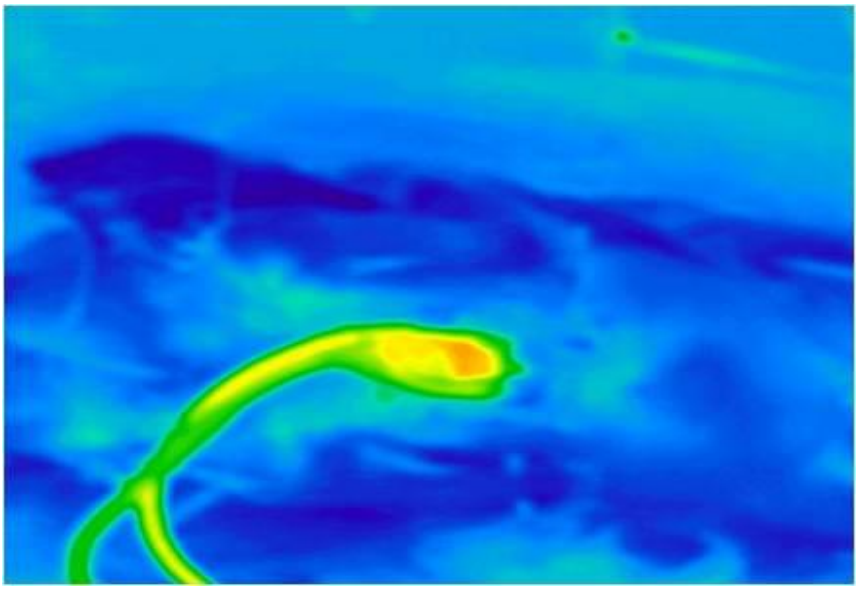
Aspects of experimental cooling and rewarming with special reference to accidental hypothermia



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Aspects of experimental cooling and rewarming with special reference to accidental hypothermia

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CONTENTS

1. ACKNOWLEDGEMENTS

This experimental work emanates from the Anaesthesia and Critical Care Research Group at the University of Tromsø, headed in these years (2001 – 2012) by always enthusiastic and supportive Lars Bjertnæs.
We started from scratch late in 2001 and went down a quite thorny road until, after more than 1 year of trial and error, we finally got our complicated pig model for deep hypothermic cardiopulmonary bypass working. However, it was not until the second series started in late 2004, with infrastructure provided by the Cardiothoracic Research Group, that data sampling became efficient.
The work was supported by a full-time grant from the Norwegian Council on Cardiovascular Diseases for 3 years from 2002. I also received financial support for technical equipment from the Norwegian Society of Anaesthesiology. After March 2005 the project has been unfunded as personal salary is concerned, and it therefore became a part-time undertaking besides my ordinary clinical work as anesthesiologist at the University Hospital of North Norway.
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2. ABSTRACT

We performed open, randomized, placebo-controlled experimental studies in intact, anesthetized pigs with the following main aims:

Paper 1.

To describe in detail the hemodynamic function with emphasis on left ventricular contractility during surface cooling, steady state severe hypothermia at 25°C, surface rewarming and in the post-hypothermic period of 2 h.

Paper 2

1) To investigate the pharmacokinetic properties of DA in normothermia and hypothermia.

2) To carry out a detailed analysis of the cardiovascular response to incrementing dosages of dopamine (DA) at core body temperatures at normothermia, at steady state hypothermia at 25°C, and during rewarming in the temperature span of 30 - 34°C .

Paper 3

To compare differences between immersion cooling and cooling by cardiopulmonary bypass (CPB) in cardiovascular function and global delivery and consumption of oxygen i) during cooling until deep hypothermic circulatory arrest; ii) during rewarming by CPB; and iii) during the 2 h post-hypothermic period following weaning from CPB.

Main results and conclusions

Surface cooling, followed by surface rewarming, resulted in a mild post-hypothermic systolic cardiac failure that was well compensated for. Diastolic function was unaffected (Paper 1).

Pharmacokinetics of DA was seriously altered at 25°C and DA did not increase cardiac output, but had the adverse effects of increased systemic vascular resistance (Paper 2).

Immersion cooling to hypothermic circulatory arrest, followed by rewarming taking place on CPB, resulted in severe and uncompensated cardiovascular failure not encountered in CPB-cooled animals (Paper 3).

3. LIST OF PAPERS

1. Filseth OM, How O-J, Kondratiev T, Gamst TM, Tveita T.
   Post-hypothermic cardiac left ventricular systolic dysfunction after rewarming in an intact pig model. Crit Care 2010;14(6):R211

2. Filseth OM, How O-J, Kondratiev T, Gamst TM, Sager G, Tveita T.
   Changes in cardiovascular effects of dopamine in response to graded hypothermia in vivo. Crit Care Med 2012;40(1):178-86

3. Filseth OM, Hermansen SE, Kondratiev T, Tveita T.
   Cooling to hypothermic circulatory arrest by immersion vs. cardiopulmonary bypass: Worse outcome after rewarming in immersion cooled pigs. Submitted

4. ABBREVIATIONS

CPB – cardiopulmonary bypass

CPB_c – animals that were cooled by cardiopulmonary bypass in Paper 3

CO – cardiac output

CPP – cerebral perfusion pressure, calculated as MAP - ICP

CVP – central venous pressure

DA – dopamine

DO_2 – global delivery of oxygen, calculated as oxygen content in arterial blood • CO

dP/dT max – maximal acceleration of pressure in the cardiac cycle

dP/dT min – maximal deceleration of pressure in the cardiac cycle

Ea – arterial elastance

Ea/Ees – arterial-ventricular coupling ratio

Ees – end systolic elastance, slope of the linear ESPVR for a family of PV-loops during VCO.

EDP – end diastolic pressure

EDPVR – end diastolic pressure volume relationship

EDV – end diastolic volume, in the present paper consisting of LV end diastolic volume and the volume of surrounding structures

ESP – end systolic pressure

ESV – end systolic volume, in the present paper consisting of LV end systolic volume and the volume of surrounding structures

ESPVR – end systolic pressure volume relationship

HCA – hypothermic cardiac arrest

Hb – hemoglobin

HR – heart rate

ICP – intracerebral pressure

IMM_c – animals that were immersion cooled in Paper 3.

LV – left ventricular

MAP – mean arterial pressure, calculated as [(2 x diastolic) + systolic] / 3

PRSW – preload recruitable stroke work , calculated as SW/EDV

PV – pressure-volume

Q_{10} temperature coefficient – a measure of the rate of change of a biological or chemical system as a consequence of changing the temperature by 10 °C. For biological systems, the Q_{10} value is generally between 1 and 3.

SR – sarcoplasmatic reticulum

SV – stroke volume, calculated as CO/HR

SVR(I) – systemic vascular resistance (index), calculated as SVR = (MAP-CVP) x 80/CO

SW – stroke work, equals area of the PV-loop

T½ – half life, the time required for a given plasma concentration of a drug to be reduced to 50 %.

Tau – a preload independent measure of isovolumetric relaxation time, based on a monoexponential decay model

TNF-α – serum tumour necrosis factor alpha

TnT – serum troponin-T

V_0 – volume axis intercept of the ESPVR slope determined by PV-loops during VCO

VCO – vena cava occlusion, abrupt occlusion of the inferior caval vein to obtain PV-loops

VF – ventricular fibrillation

VO_2 – global consumption of oxygen, calculated as difference of arterial and mixed venous oxygen content x CO

5. INTRODUCTION

5.1 Classification of hypothermia

Hypothermia describes a state in which the body's mechanism for temperature regulation is overwhelmed in the face of a cold stressor. Hypothermia is classified as unintentional or intentional (1). Unintentional primary hypothermia, or simply accidental hypothermia as will be used in the present thesis, is due to environmental exposure, with no underlying medical condition causing disruption of temperature regulation. Unintentional secondary hypothermia is low body temperature resulting from a medical illness lowering the temperature set-point or hypothermia secondary to major trauma. Iatrogenic hypothermia in the operating theatre can also be classified as unintentional secondary hypothermia. Intentional hypothermia encompasses protective hypothermia utilized in surgery on the heart and great thoracic vessels, and therapeutic hypothermia used to mitigate brain damage after successful resuscitation from cardiac arrest. The classification of hypothermia is summarized in Table 1.

Cause	Circumstance
Unintentional hypothermia	
Accidental (primary)	Environmental exposure
Medical illness or trauma (secondary)	Lowered set-point; major trauma and burns
Intentional hypothermia	
Protective	Surgery on heart and thoracic vessels
Therapeutic	Post-resuscitation after heart arrest.

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Table 1. Classification of hypothermia

In the present thesis the main focus will be on accidental hypothermia. However, as discussed in the "Aims of the thesis" section our methodology and some of our results also have relevance for therapeutic and protective hypothermia.

5.2 Ranges of the severity of hypothermia

Different nomenclatures for the severity of hypothermia exist. Popovic (1974) suggested mild above 32°C, moderate 22-32°C, deep 8 – 22 and profound 0 - 8°C (2). Wong (1983) made use of mild 32-35°C, moderate 26 - 31°C, and deep 20 - 25°C (3), while Moss (1986) applied mild 32-35°C, moderate 28-32°C, and severe below 28°C (4), as does the European Resuscitation Council (2010) (5) .
American Heart Association (AHA), however, makes use of mild hypothermia above 34°C, moderate 30-34°C, and severe below 30°C (6). Polderman and Herold (2009) apply the same distinction, only substituting "deep" for "severe" for temperatures below 30°C, as their topic is therapeutic/protective hypothermia (7).
For clinical reasons, we think that the AHA definition is the simplest and most operational one, appointing 30°C as an important "watershed" temperature marker between "safe" moderate (30 - 34°C), and potentially life threatening severe (below 30°C) hypothermia.

Therefore, in the present thesis the following definition will be used:

Mild hypothermia: 34 - 35°C
Moderate hypothermia: 30 - 34°C
Severe (or *deep* when applied in protective hypothermia) hypothermia: Below 30°C.

It is important to note that the severity of unintentional hypothermia is far more dramatic in hypothermia secondary to trauma than in accidental (primary) hypothermia. While victims of accidental hypothermia usually have a good prognosis if body core temperature is above 30°C, a 100% mortality has been reported in major trauma patients presenting with a body core temperature below 32°C (8), and hypothermia has been found to be an independent risk factor for mortality in major

trauma (9). As a consequence, alternative ranges of the severity of hypothermia secondary to trauma to those used in accidental hypothermia have been proposed (10).

5.3 Brief historical overview of accidental hypothermia

One way to justify research projects is to state that the pathological condition in study "is a major killer". Tempting as it may be, there is no evidence that accidental hypothermia has ever been one of mankind's scourges. Even in arctic societies, there is no documentation that incidence of accidental hypothermia has been higher than in more temperate climate zones. The reason for this is obvious: Indigenous peoples in circumpolar regions have adapted to the cold, both through sociocultural practises, long-term natural selection and individual adaptation. Anyone having read Norwegian adventurer Helge Ingstad's account of his life among caribou-eating Canadian Indians in the 1920'ies will remember how he managed to survive winters with air temperatures as low as -60˚C by adopting Indian clothing and a diet consisting of 100% caribou without any additives (11).

However, as this is written, the 8th of February 2012, news agencies report that more than 420 people, mostly poor, homeless and from Central and Eastern Europe, have died from extreme winter cold in recent days. It is likely that down-scaling of social services following globalization and financial crisis will render more people world-wide in poverty and despair. In combination with extreme weather due to climate changes these political conditions will probably lead to increasing numbers of deaths from "urban" hypothermia in the future.

5.3.1 Accidental hypothermia in war

When masses of people have been forced into un-physiological and absurd situations in the service of war, accidental hypothermia has killed along with steel flying through the air, hunger and epidemics. Numerous accounts of war-related accidental hypothermic mass death exist. Carthaginian general Hannibal lost 23000 men, probably to a large extent due to hypothermia, in a winter crossing through the Alps in 218-217 B.C. in an attempt to conquer Italy (12). General Armfeldt, subordinate to Swedish king Charles 12th and leader of a campaign in Norway in 1718 – 1719 during

the Great Nordic War, ordered his troops to withdraw after the king was killed. His army tried to cross the mountains at Tydal in Norway in January 1719, with the result that nearly 5000 soldiers froze to death in severe snow storms (13). Both Napoleon and Hitler were eventually stopped by the cold hostile Russian winter for which their troops were not prepared in their attempts to conquer Moscow.

5.3.2 Accidental hypothermia in peace time

In peace time, mass death from hypothermia has been linked to shipwrecks. Most referred to is the story of the "unsinkable" steamship Titanic that hit an iceberg on her virgin voyage in 1912. It is believed that cold water and inappropriate clothing killed most of the around 1500 people that died in the disaster (14). More recently, the potential for hypothermic mass death was present when cruiseship SS Maxim Gorkij hit an ice floe near Svalbard in 1989. Around 1000 passengers, many of whom were elderly and wearing only their nightclothes, were evacuated to surrounding ice floes, from where they luckily could be saved by Norwegian Coast Guard some hours later (15). A similar incident took place in Antartica in 2007 when around 150 people were evacuated to lifeboats from the sinking cruiseship MS Explorer. Due to calm weather all could be rescued around 5h later by the Norwegian coastal steamer MS Nordnorge (16).

5.3.3 Incidence of death from accidental hypothermia

No national registry for deaths from primary accidental hypothermia (besides general death certificates) exists in Norway. In a review article from 1986 it was referred that 411 deaths from accidental hypothermia was registered from 1945 to 1979, but that this number may be misleading, as some of the 200-250 people categorized as dead by drowning annually in fact may have been victims of accidental hypothermia (17). Based upon own experiences at the University hospital of North Norway (population about 500.000) extracorporeal rewarming after accidental hypothermic circulatory instability or arrest is undertaken in 3 – 5 patients every year. Of these, snow avalanche victims usually die. In addition, in an odd year a person is declared dead from hypothermia without being submitted to any hospital. If these numbers reflect the

real incidence in the country it could be estimated that the death rate from accidental hypothermia in Norway amounts to about 0.5 deaths per 100.000 inhabitants per year. This may be in concordance with numbers from other countries in the Northern hemisphere, where deaths from accidental hypothermia per 100.000 inhabitants have been reported to be 0.13 in France (18), 0.3 in the USA (19) and 2 in Great Britain (20).

Even if deaths from accidental hypothermia may be rare compared with other causes of death, hospitals in Norway must be prepared to treat a predictable number of hypothermic casualties every year. With increasing activity in subarctic and arctic regions in the form of cruise ship tourism, establishment of new circumpolar oil and gas fields and increased transcontinental shipping following north pole ice melt-down, scenarios of mass hypothermic injury must be incorporated in regional and national emergency operation plans.

In section 5.5.3 prognosis of accidental hypothermia and causes of death will be discussed.

5.4 Brief historical overview of hypothermia research

5.4.1 Research prior to the second world war

As mankind's history of accidental hypothermia on a large scale is closely linked to warfare, it is not surprising that great wars combined with the technological development during and after the industrial revolution served as catalysts for hypothermia research. As a consequence of the decimation of Napoleon's armies by the cold during their retreat from Moscow and the futile attempts at rewarming them, extensive research on the physiological effects of cooling were performed by French physiologists around the 1850'ies (21). During the first world war 12.000 Royal Navy sailors, 10.000 merchant seamen and 5.000 German sailors drowned (22). Strangely enough, this dreadful statistics did not spur any research efforts to address the cause of death and the possible role of hypothermia in the relatively short time span of interwar peace (22).

5.4.2 The Nazi hypothermia experiments and implications for later hypothermia research

With the onset of the second world war the Germans first saw the need for hypothermia research. Of 40.000 men in the German submarine service about 70 % lost their lives, many of whom possibly by hypothermia (23). In the air battle with Britain, hundreds of pilots from Luftwaffe were shot down over the cold North Sea. Some casualties were rescued alive, only to die shortly after rescue. At that time the concept of core temperature afterdrop was unknown, as was the cause of the circum-rescue collapse (23;24). There was no scientific literature describing human responses to immersion hypothermia, nor information as to the safety and efficacy of various rewarming strategies.

In such a context the Nazi leadership initiated the infamous hypothermia experiments in the concentration camp in Dachau in 1942. Part of this context was also, even if under-communicated by the victorious nations of the second world war, that use of prisoners for experiments was commonplace. In fact, various dangerous and inhumane medical experiments on criminal prisoners flourished in the USA during the second world war and expanded tremendously after the war, seemingly unaffected by the Nurnberg trials that convicted many German researchers to death penalties, until the final shut-down of prison experiments in the 1970'ies (25).

A central question after the second world war has been whether data generated from hypothermia experiments on unwilling concentration camp prisoners in Dachau should be utilised. While the existence of these data cannot be ignored, it is possible to look into this dark chapter of medical research without giving the data generated from the experiments any scientific benchmark status, as demonstrated by Pozos (23).

Some reviewers simply reject data from the Dachau hypothermia experiments on grounds that they were poorly designed from a scientific point of view, and conducted by criminal outcasts in the German medical society (26). Pozos convincingly contradicts this view: The Dachau experiments were not designed as scientific research *per se*, but to find practical solutions to an urgent problem of national significance. Besides, modern use of statistics had at that time not permeated medical research, and lastly; Dr. Rascher, who was responsible for conducting the experiments,

was supervised by acknowledged medical authorities (23).
The data from the hypothermia experiments in Dachau have been extensively used in medical literature. From ethical and political reasons, we should not use them, as we should abstain from the use of data from abundant inhumane prison experiments in the USA and elsewhere in the world in other research areas. However, we should not forget that these experiments have taken place. As quoted from a letter from one of Pozos' colleagues: "When human beings are given differential value then we are all vulnerable. The Dachau data is really irrelevant. What is relevant is medicine and science's placing differential value on human life. If we permit the continued acceptance of the consequences of that evil, then we are all at risk" (23).

5.4.4 Hypothermia research after the second world war

After the end of the second world war it was estimated that 20 – 30.000 men serving in the British Navy had died (22). One third was killed in action and two thirds had principally drowned due to the cold during the survival phase. The reported deaths precipitated an extensive research programme facilitated by the British Navy, and resulted in numerous publications and reports (22). In the continuation of this research programme researchers like F.S. Golden and M. J. Tipton have specialized within the field of accidental immersion in cold water, hypothermia-induced failure of swimming as a cause of drowning and the phenomenon of circum-rescue collapse (22;24)
After the second world war researchers outside the military realm, especially in North America, started to conduct cooling and rewarming experiments on intact animals, mostly dogs. Aims of studies were to disclose cardiovascular changes and oxygen metabolism during cooling and rewarming in addition to factors determining cooling rates (27-29). Their focus was not primarily treatment of accidental hypothermia, but rather general physiological principles. The practical spin-off would be the utilisation of hypothermia as a protective measure in heart surgery (29). During the following years similar experiments were performed to examine the effects of hypothermia on circulating blood volume (30-32) and the effects of short vs long periods of hypothermia on the cardiovascular system (33-35). By the end of the

1950'ies and the beginning of the 1960'ies more sophisticated methods were developed and used in hypothermia experiments, like application of strain gauge arches to assess myocardial contractility in intact animals (36;37), and isolated perfused heart preparations for the assessment of intraventriclar pressure-volume (PV) loops (38). The effects on the cardiovascular system of cathecholamines like epinephrine, nor-epinephrine and DA in hypothermia were also tested in intact animal models (39-42), as is further described in section 5.6.
Examples of experimental hypothermia research areas in the last decades include Austrian porcine studies of hypothermia combined with vasopressors during cardiopulmonary resuscitation (43), studies on deep hypothermic circulatory arrest (HCA) in Oulu, Finland (44), research on trans-capillary fluid shifts during hypothermia in Bergen, Norway (45) and cooling-rewarming studies on dogs and rats to disclose post-hypothermic cardiovascular failure in Tromsø, Norway (46;47).

5.4.5 Clinical hypothermia research

When it comes to clinical hypothermia research, numerous studies have been performed within the fields of protective and therapeutic hypothermia. A search for "therapeutic hypothermia" in the Cochrane data-base per December 2011 revealed 266 prospective, randomized clinical studies. When searching for "accidental hypothermia", only four relevant clinical studies met the Cochrane criteria: Peripheral rewarming using a charcoal-fuelled heating device inside a sleeping-bag and inhalation rewarming proved inefficient in volunteers that were cooled to 35˚C (48); active external rewarming by forced air speeded rewarming in hypothermia victims (49); anesthetized volunteers rewarmed faster by active than by passive external rewarming (50), and motion sickness increased cooling rate in volunteers (51).
The majority of clinical reports on accidental hypothermia are in the form of case stories. By meta-analysis some general insights may be drawn from these stories. Tipton and Golden recently collected 43 reports of victims world-wide who had survived prolonged submersion in water (52). No records reported survival if the victim had been submersed for more than 30 minutes in water warmer than 6˚C. Also, only one of 43 surviving casualties was retrieved from saline water (52).

There are some retrospective clinical studies analyzing rewarming from accidental hypothermia, with a reported mortality ranging from around 30 to 80% (18;53-56). In a recent retrospective study of 84 victims of accidental hypothermia from Amsterdam, the Netherlands, in their conclusion the authors point to a core problem in clinical research in accidental hypothermia: "Accidental hypothermia is a rare diagnosis in an inhomogeneous population, treated with a large variety of rewarming techniques. (…) Because individual teams gain little clinical experiences, we suggest multiple centre data collection as a first step towards an evidence-based standard of care" (56). Indeed, some of the authors of the study have collaborated with other capacities within the field to launch an international prospective registry, where data of individual cases of accidental hypothermia all over the world can be registered in a standardized way (57).

5.4.6 Research in the era of molecular biology, genomics and computer simulation: Is the intact animal model obsolete?

In experimental research, ever more sophisticated techniques have been used to isolate ever smaller structures. In 1960 Monroe and French isolated the dog heart to extract volume-pressure relationships and myocardial oxygen consumption (58). While similar set-ups are still commonly used, modern research now also deals with sub-cellular structures down to the molecular level, for instance to explain excitation-contraction coupling in cardiomyocytes (59). Such insight is crucial to understand basic physiology of heart function. Still, cardiovascular function in intact organisms cannot be predicted from knowledge of the function of sub-cellular parts, let alone complete organs. As an example, in recent years an integrated view of the interaction between vascular tone and cardiac contractility has been pursued through the study of the coupling between arterial and cardiac elastance (60;61). The significance of an reduced systemic vascular resistence after rewarming from deep hypothermia is not necessarily a malfunction of the vasculature, but may be seen as an adaptive measure to a post-hypothermic cardiac failure (62). The combined effect of hypothermia itself and the associated low-flow circulation on blood viscosity (63;64), is another factor that contributes to the in vivo complex cardiovascular responses to hypothermia that

would be nearly impossible to simulate or deduct from the study of single cells or organs.

Another common topic in modern biological and medical research is the study of genetics. The mapping of mammalian genomes should in theory be helpful in designing experimental models. For instance, in the study of catecholamine effects in hypothermia, it might be useful to know that human and porcine subtypes of α-adrenoceptors are genetically identical and may differ from homologous rodent subtypes (65). Likewise, it could be considered a limitation for a pig model that there exists a species-dependent selectivity of agonists to a subtype of β-adrenoceptors that distinguish primates from other mammals (65). However, genomics is not simple mathematics: In the majority of organs the adrenoceptors expressed there do not correspond to the functional roles they play, also it has been demonstrated that a subtype of α-adrenoceptors is non-functional at normothermia, but becomes functionally predominant at lower temperatures (65). To map the genetic expression of adrenoceptors in various organs in different species would not predict species-specific responses to exogenous catecholamines in normothermia nor in hypothermia. It seems that we would still have to test and observe using established physiological methods.

5.5 Cardiovascular failure and prognosis in accidental hypothermia

5.5.1 Experimental studies of the effect of hypothermia on cardiac function

Research results have been somewhat confounding regarding the effect of low body temperature per se on myocardial function. From studies on isolated dog and rabbit hearts subjected to moderate and severe hypothermia, increased left ventricular (LV) contractility and increased cardiac work have been reported (38;66). Core cooling to 33°C in a pig model mimicking therapeutic hypothermia suggested improved systolic, but depressed diastolic function (67); similar results were found in surface cooled dogs (68).

In severe hypothermia, increased LV contractile force was demonstrated in intact dogs during surface cooling to 20 to 25°C (37). Likewise, immature swine cooled by extracorporeal circuit peaked in LV stroke volume (SV) and work at 29°C (69). On the

other hand, intact dogs that were core cooled to 25°C and rewarmed showed reduced myocardial contractility during as well as after hypothermia (70). There is experimental evidence that long time exposure to severe hypothermia leads to a specific non-ischemic cardiac failure (35;47;70-72) that is related to intracellular accumulation of calcium in cardiomyocytes (73).
The issue of differences in physiologic effects between species was demonstrated in a recent comparative study using cardiac tissue from humans and rabbits that revealed reduced inotropy by moderate hypothermia in human as opposed to rabbit (74). The findings were related to differences in myocardial tissue sarcoplasmic reticulum Ca^{2+} storage and Ca^{2+} sensitivity (74).

5.5.2 "Rewarming shock"

The clinical term "rewarming shock" has been applied to describe the observation of hypotension and low cardiac output (CO) during rewarming (75). Maclean and Emslie-Smith (1977) make use of the synonymous term "rewarming collapse" as resulting from a sudden fall in peripheral resistance unmatched by a compensatory increase in CO, frequently encountered in elderly patients in the rewarming phase (76). Kuehn (1983) alternates between "rewarming collapse" and "rewarming shock". The proposed mechanism is intravascular hypovolemia during rewarming, as well as peripheral vasodilation and possibly release into the circulation of cold, stagnant blood (77). There seems to be an unison view that rewarming has to be accompanied by infusion of warm fluids to avoid hypovolemia (21;76;78), and that inotropic support may be added when core body temperature exceeds 30˚C if CO is estimated to be inappropriately low despite adequate volume substitution (79). It can be questioned whether rewarming collapse or rewarming shock describe a frequently encountered clinical syndrome, or if the terms merely point to a combination of transient cardiac failure, peripheral vasodilatation and reduced circulating blood volume that may or may not appear in the rewarming phase. As will be discussed in the following section, cardiovascular collapse during rewarming from accidental hypothermia does not seem to be a major clinical problem in contemporary medicine.

5.5.3 Prognosis of accidental hypothermia and causes of death

In retrospective studies of accidental hypothermia patients admitted to hospitals, there is a general tendency that older studies (from the 1970'ies and -80'ies) report a high mortality, according to Vassal et al between 52 and 80% (18). In their own institution in Paris, Vassal et al reported an overall mortality of 38 % in 65 cases of "urban hypothermia" (≤ 32˚C) that were admitted over a long period (1979 – 1998) (18). Other European studies report a mortality of around 30% (53;56). Low blood pressure on admission (18), slow cooling (53), slow rewarming (18;53), submersion and asphyxia (53;56), old age (18;53;56), indoor exposition and low temperature (56) were negative prognostic factors, whereas intoxication by alcohol or narcotics favoured survival (18;53). In the most recent of these studies, van der Ploeg et al (2010) report that of 84 hypothermic patients the majority of non-survivors did not die during rewarming, but from late multi-organ failure especially involving kidney, liver and the coagulation system (56).

Clearly, prognosis in accidental hypothermia is better if patients present with spontaneous circulation and if the admitting institution has a rewarming protocol. Thus in Innsbruck, Austria, it was reported that 15 victims of severe accidental hypothermia presenting with a spontaneous cardiac rhythm were rewarmed safely and efficiently by forced air (54). All patients survived rewarming, however the 6 patients that had prehospital cardiac arrest (all were resuscitated to ROSC at admission) did not survive long-term, mainly because ischemic brain damage after submersion or avalanche accidents. The remaining 9 patients had excellent long-term recovery (54)

Even if hypothermic patients present with circulatory arrest, or un-stable spontaneous circulation at admission, prognosis can be fair if asphyxia did not precede hypothermia, and outcome can be excellent even in the most extreme cases (80;81). In Bergen, Norway, Farstad et al (2001) collected 11 records of patients belonging to this category, of whom 7 survived without neurological deficit (55). No surviving patient had a serum K^+ value that exceeded 10 mmol/L. 14 of 15 patients with asphyxia (drowning or avalanche) preceding hypothermia died, mainly due to irreversible brain damage (55).

It may seem that in modern day intensive care, the major causes of death from

hypothermia are co-morbidity and late multiorgan failure, including irreversible ischemic brain damage resulting from asphyxia that preceeded the hypothermic insult.

5.5.4 Circum-rescue collapse

The term "rewarming collapse" may be confounded with the concept of circum-rescue collapse, which is applied especially to the rescue of people from water (22;24). Circum-rescue collapse implies sudden cardio-vascular derangement and sometimes cardiac arrest, not from low body core temperature per se, but from alteration in intravascular blood volume distribution during extraction from water and possibly by altered blood catecholamine levels at the prospect of rescue (22;24).

5.5.5 "Afterdrop" as explanation of hypothermic cardiovascular failure

The term "afterdrop" describes a fall in core temperature which occurs immediately after the surface cooled patient is removed from cold surroundings (17).
Afterdrop was originally believed to be caused by cold venous blood returning centrally from cooler peripheral tissues (21) and to be aggravated by active surface rewarming that was believed to cause peripheral vasodilatation and pooling of cold, stagnant blood to the heart, thereby inducing cardiac failure and dangerous arrhythmias (77). Consequently, core rewarming was considered superior to surface rewarming to avoid afterdrop (77). However, afterdrop has been explained by direct conduction alone (82), especially since it was also observed in dead pigs (83), and the circulatory component in live animals and volunteer humans has been considered insignificant (21;84).
Afterdrop at a rate of 1.4 – 4.0° C/h has been reported in avalanche and immersion victims that present with mild or moderate hypothermia at the time of excavation from the snow or extraction from water (85;86). Rescue and primary therapy should aim at reducing afterdrop and other circum-rescue risk factors. There is however no evidence that core rewarming is superior to surface rewarming in avoiding afterdrop, or that deliberately slow surface rewarming (for example by keeping limbs during initial rewarming

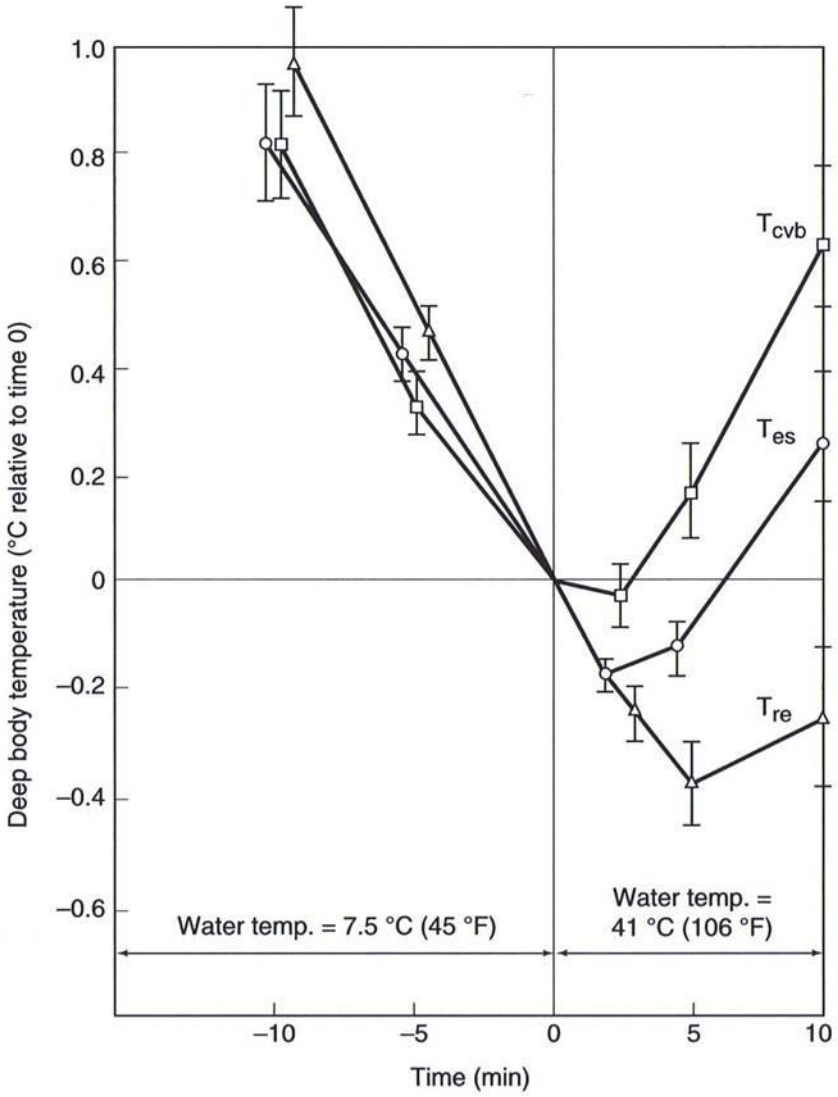

Figure 1. **Afterdrop.** Mean changes in temperatures in central venous blood (T_{cvb}), oesophagus (T_{es}) and rectum (T_{re}) in 24 anesthetized live pigs cooled to 31˚C, and put directly into a tub with hot water. *From F.S. Golden (1983)*

5.6 Effects of catecholamines and vasoactive drugs in experimental hypothermia

Different catecholamine and vasoactive drug regimens have been trialled in experimental hypothermia.

In pig models of HCA both epinephrine and vasopressin led to increased coronary perfusion pressure during cardiopulmonary resuscitation (87), but epinephrine did not result in more successful return to spontaneous circulation (88) and vasopressin did not increase short time survival (89).

The following section deals with models involving a perfusing (spontaneous) circulation during hypothermia.

5.6.1 Endogenous catecholamine responses during hypothermia

In awake homoeothermic animals exposure to cold that may lower body temperature is stressful and will lead to strong neuroendocrine activation evident from increase in plasma concentrations of epinephrine and norepinephrine, and an increase in heart rate (HR) and blood pressure (90). Warner et al (1970) subjected dogs anesthetized by ether or halothane to profound hypothermia at 15°C by internal cooling (41). Apparently the anesthetic level was not deep, as cooling produced an increase in plasma levels of epinephrine and nor-epinephrine. 2/3 of the dogs had ventricular fibrillation (VF) at a core temperature of 15°C. When the dogs received propranolol or compound P-286, a drug thought to prevent release of catecholamines from the adrenal gland, no animals had VF at this temperature (41). This suggests a possible undesirable effect of sympathetic activation in eliciting VF during cooling.

There is experimental evidence that the stress reaction of cooling may eliminate the positive effects achieved by applying therapeutic hypothermia (91;92). As a recognition that it is the combined effect of sedation/anesthesia and hypothermia that may favor both the central nervous and the cardiovascular system after ischemic damage caused by cardiac arrest, deep sedation is now an integral part of therapeutic hypothermia protocols (7).

5.6.2 Effects from administration of epinephrine, nor-epinephrine and dopamine

Simulation of sympathetic activation during cooling is possible in anesthetized animals by administration of exogenous catecholamines. Kondratiev and Tveita (2006) demonstrated a significant posthypothermic myocardial depression in rats treated with epinephrine during cooling compared to temperature-matched controls (93). Epinephrine administered during rewarming also had negative effects: Epinephrine in a dose that induces vasodilatation and elevated CO in normothermia resulted in vasoconstriction without elevation in CO when administered during rewarming from 24°C (94). The posthypothermic CO and left ventricular SV was reduced by 30 % in rats treated with epinephrine during rewarming compared to temperature matched controls (94).

The findings of Kondratiev et al parallel those of Rubinstein (1961) that a defined

dosage of epinephrine given to dogs caused vasodilatation in normothermia, but increased total systemic vascular resistance (SVR) at 25° C (95).
The arrhythmogenic effect of exogenous epinephrine during cooling was demonstrated by Angelakos and Daniels (1969), who surface cooled anesthetized dogs until a terminal temperature when VF or asystole occurred (42). Epinephrine infusion increased the incidence of VF from 60 to 100% and increased the terminal temperature from 19,3 to 21,9°C.
While some unfavourable effects of epinephrine in experimental hypothermia have been demonstrated, most studies so far involving DA in the same setting could give the impression that DA is a more appropriate drug. In the study by Angelakos and Daniels described above DA had a seemingly protective effect against VF, since infusions of DA (and nor-epinephrine) reduced the incidence of VF from 60 to 20 % and the terminal temperature from 19,3 to 12,5°C (42).
Nicodemus et al (1981) demonstrated that a DA infusion at 12 μg/kg/min during internal rewarming of anesthetized dogs from 25°C reversed cold-induced cardiovascular depression without causing ventricular arrhythmias (96). Oung et al (1992) reported that optimal dosage for DA to improve CO in pigs at different core temperatures (30 - 38,5°C) was 10 – 20 μg/kg/min and that no arrhythmias occurred except for sinus tachycardia at dosages up to 30 μg/kg/min (97). In a study of lung-transplanted pigs that were immersion cooled to 32°C DA in dosages of 5 and 12 μg/kg/min decreased mean arterial pressure (MAP), but increased cardiac index (CI) and had no effect on pulmonary vascular resistance (98). A negative side effect of DA during hypothermia was reported as a DA-dependent increase in left ventricular end-diastolic pressure (LVEDP) in anesthetized sheep core cooled to 29°C (99).

5.7 Clinical use of catecholamines in hypothermia

Due to lack of human studies, the recommendations for the clinical use of catecholamines in accidental hypothermia are based on animal experiments or clinical experience and corollary the opinions have been somewhat differing. Wong (1983) postulated that positive inotropic cardiac effects of catecholamines are enhanced during mild to moderate hypothermia and depressed at deep hypothermia (3). Danzl

(1994) stated in general terms that target organs and sytems become progressiveliy less responsive to medications as the core temperature falls (100), similar to the view of Lloyd (1996), who claimed that the hypothermic heart is unresponsive to pacing and cardioactive drugs (82).

In general, both European and North American guidelines advice against inotropic or anti-arrhythmic drugs at core temperatures below 30°C, the reason being that these drugs are considered ineffective in deep hypothermia and may accumulate to toxic quantities (5;6). According to these guidelines drugs may be used at body core temperatures above 30°C but with increased interval between doses. Mechem and Danzl (2008) advocate the use of a low-dose infusion (2 to 5 microg/min) of DA in victims of deep or moderate hypothermia when the circulation is estimated to be unsustainable in spite of adequate fluid resuscitation (79), a recommendeation that is based on experimental studies on pigs and sheep referred to above (97;99).

Even if written guidelines for the use of inotropic medication at reduced core temperatures are scarce and based on a limited number of preclinical studies it has been reported that a majority of hypothermic patients receive inotropic drug therapy when treated with moderate therapeutic hypothermia after resuscitation from cardiac arrest (101;102), or during rewarming from accidental hypothermia (18;54). In contrast, in patients hospitalized for acute heart failure without hypothermia, a subgroup of about only 10% received inotropic drugs (103).

5.8 Pharmacology of hypothermia

The knowledge of the effect of hypothermia on the pharmacology of various drugs seems relatively sparse (107). Tortorici et al (2007) reviewed twenty-one studies on hypothermia-mediated alterations on the cytochrome P450 enzyme system (108). Among the drugs studied were opiates, benzodiazepines, barbiturates and nevromuscular blockers. They found that mild to moderate hypothermia in humans decreased the systemic clearance of the drugs between ~7 % and 22 % per degree Celsius below 37°C and that hypothermia decreases the potency and efficacy of certain drugs. As catecholamines are metabolised by uptake in nerve endings and by the

enzymes monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) they were not the aim of study. In their review article, Pedersen et al (2007) likewise do not deal with catecholamines other than citing an article by Cotton et al from 1957 that demonstrated attenuated effect of nor-epinephrine on contractility and arterial blood pressure during hypothermia in an experimental setting (109).

5.9 Clinical rewarming from accidental hypothermia

Various techniques in rewarming victims of accidental hypothermia have been described: Passive, or spontaneous rewarming, relying on endogenous heat producton and facilitated by insulation (for example carpets) and warm surroundings, and active rewarming where heat is conveyed to the victim. Active rewarming takes place by surface rewarming, or by core (internal) rewarming. Surface rewarming can take place by immersion in warm water, by packing in warm blankets, by charcoal-driven devices inside sleeping bags and by forced warm air (19, 48, 49, 82). Several forms of active core rewarming have been proposed for hypothermic victims: Airway warming, irrigation of body cavities (mediastinum, pleura, peritoneum), intragastric, intraoesophageal and intracolonic devices, intravenous fluids, and cardiopulmonary bypass (19,82). However, there is support in the literature that intra-hospital management of hypothermic victims can be substantially simplified: Victims of accidental hypothermia presenting with what is judged as sustainable, spontaneous circulation can be successfully surface rewarmed by forced air irrespective of the core body temperature on admission (54). If circulation is judged unsustainable (asystole, ventricular fibrilliation or other serious arrhythmias; severley reduced cardiac output) rewarming by cardiopulmonary bypass (CPB) is the preferred method, both in adults and children (55, 123, 124).

While the controlled cooling by CPB until deep HCA at 15 – 18°C to perform complex aortic surgery followed by rewarming results in an overall hospital mortality of only around 5% (111, 112), hospital mortality of victims of accidental hypothermia that are rewarmed by CPB has been reported to be as high as 70 – 87% (55, 110).

6. AIMS OF THE THESIS

The studies that the present thesis is based upon were initiated in 2003, highly inspired by the successful and sensational resuscitation of a young woman that was admitted to our institution with a core temperature of 13.7˚C (80).

In developing an intact pig model to study the whole time-course of cooling to severe hypothermia, followed by steady state hypothermia, rewarming and post-hypothermic expiration, we have revisited hypothermia experiments initiated by the pioneers 60 years ago.

The reason for undertaking Paper 1 was the somewhat confounding results from earlier experimental hypothermia research regarding low body temperature *per se* on myocardial and vascular function (62). Among questions to be raised and discussed are:

- Is hypothermia-induced heart failure after rewarming from deep hypothermia characterized by both systolic and diastolic failure, as seen in myocardial stunning?
- Do post-hypothermic changes in SVR express adaptive measures or compensatory incapacitation?

Paramount in the effort to answer these questions was the use of an indwelling conductance catheter in the left cardiac ventricle to extract pressure-volume and contractility data.

Background for the study in Paper 2 was the discrepancy between the limited knowledge about pharmacologic effects and pharmacokinetic properties of catecholamines in hypothermia, and the widespread use of catecholamine drug therapy in patients undergoing moderate therapeutic hypothermia (101;102) or rewarming from accidental hypothermia (18;54).

As in Paper 1, Paper 2 makes use of the conductance catheter as a crucial tool to study the interaction between the heart, the vasculature and the circulating blood volume under the influence of DA at various core body temperatures.

In Paper 3, we would try to address the clinical experience that extracorporeal rewarming followed by intensive care of victims of severe accidental hypothermia seems much more complicated than the routine rewarming by CPB of patients

undergoing deep protective HCA for surgery on the proximal aorta (55;110-112). Would there be any difference in outcome if the only difference between the experimental groups was the cooling method; surface cooling vs. cooling by cardiopulmonary bypass (CPB)?

Accidental hypothermia has remained the main aim in the present thesis. However, as will be discussed later, our methodology and some of our results also have relevance to therapeutic and protective hypothermia.

Specifically, the aims of the papers are as follows:

Paper 1.

The aim of paper 1 was to develop a minimally invasive, closed chest pig model to describe in detail the hemodynamic function with emphasis on left ventricular contractility during surface cooling, steady state severe hypothermia at 25°C, surface rewarming and in the 2 h post-hypothermic period.

Paper 2

The aim of paper 2 was to use the model established for paper 1 to 1) carry out a detailed analysis of the cardiovascular response to incrementing dosages of DA at core body temperatures at normothermia, at steady state hypothermia at 25°C, and during rewarming in the temperature span of 30 - 34°C; and 2) investigate the pharmacokinetic properties of DA in normothermia and hypothermia. Also, we investigated 3) whether substantial doses of DA given during hypothermia would blunt the post-hypothermic effects of DA, and 4) the effect of DA on the rate of rewarming.

Paper 3

The aim of paper 3 was to compare differences between immersion cooling and cooling by CPB on 1) cardiovascular function and global delivery and consumption of oxygen and 2) brain perfusion pressure and brain metabolism in an intact pig model in different phases: i) during cooling until severe/deep HCA; ii) during rewarming by CPB; and iii) during the 2 h post-hypothermic period following weaning from CPB.

7. METHODOLOGICAL CONSIDERATIONS

In the description of material and methods the names and addresses of producers of various equipments are largely omitted to enhance readability. In the original papers that are attached this information is included.

Descriptions of standard hemodynamic recordings and calculations, as well as biochemical analyses are made rather short and straight-forward. When it comes to more specific methods that were crucial to our models the accounts have been made more detailed.

7.1 Animals

Previous experimental hypothermia studies of surface cooling/rewarming in animals with maintained circulation have been performed using rodents (34;47) and dogs (33;113). However, cardiovascular responses to hypothermia in these species may differ from humans since rodents increase their SV during severe hypothermia (47;94), whereas in dogs, whose SV remain unchanged at this temperature zone, an elevated SVR is maintained after rewarming even from prolonged surface hypothermia (33;113), contrary to what has been reported from the clinical rewarming in accidental hypothermia (76, 77).

The apparently closer morphologic and physiologic relationship between humans and pigs suggests that a porcine model is more suitable for translational research (114). There were also other reasons for selecting the pig as model animal: In Papers 1 and 2 we utilized an indwelling conductance catheter in the left ventricle, a technique established by other researchers in pig models at our lab (115;116). In Paper 3, we used CPB in the control group during cooling, and on animals in both control - and study groups during rewarming. In this respect, we could lean on other researchers at our lab who have applied normothermic CPB in porcine models for many years. We also visited Finnish researchers led by T. Juvonen that have an extensive experience in chronic porcine models of deep HCA lasting 75 min before rewarming and recovery (44).

The animals used were 2 – 3 months old castrated male pigs weighing 24 - 37 kg. The breeds were either a native Norwegian stock (norsk landsvin) (Papers 1 and 2), or a hybrid crossing of native Norwegian and British Yorkshire breeds (Paper 3).

7.2 Ethics

Experimental protocols were approved by the local steering committee of the Norwegian Animal Experiments Authority. The animals received humane care in accordance with The Norwegian Animal Welfare Act. After arrival at the laboratory animal unit they were placed in pens for 2 – 5 days, where they were fed twice daily and had free access to water at all times.

During experiments, the animals were deeply anesthetized. The anesthesia protocol was similar in all series. After termination of experiments animals were killed with 20 mmol potassium chloride given as an i.v. bolus. No neuromuscular blockers were used at any time.

Fig. 2 (analyses from animals in Papers 1 and 2) demonstrates that no increase in the stress hormone epinephrine was detected throughout experiments. For comparison, I have included Fig 3 showing the stress response of surface cooling without use of anesthetics in an animal study from the USA (90).

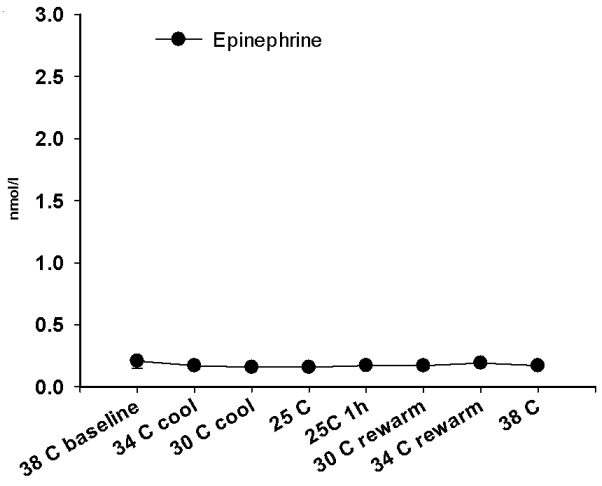

Figure 2. Plasma concentrations of epinephrine during cooling and rewarming in deeply sedated pigs in the present studies.

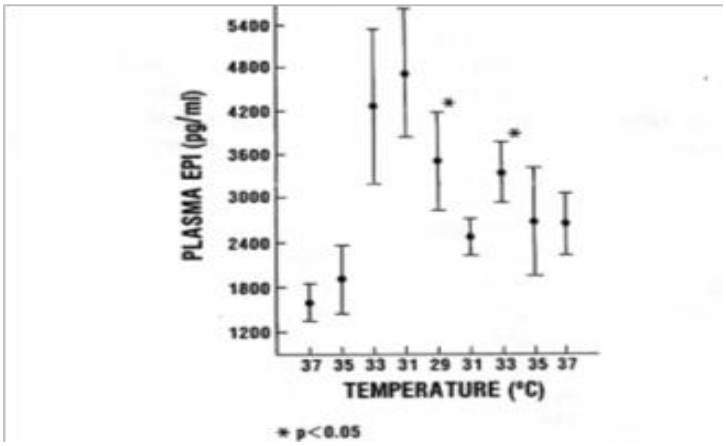

Figure 3. Plasma concentrations of epinephrine during cooling and rewarming in awake baboons. *From Chernow et al (1983).*

7.3 Study design

All studies were performed as acute, prospective, open, placebo-controlled experimental studies in the animal research laboratory affiliated to the University of Tromsø, Norway. In Papers 2 and 3, randomisation between study- and control groups was also performed.

In Paper 1, 8 animals were surface cooled and kept at 25˚C core temperature for 1 h before rewarming. Comparisons were made "within group", or "between groups" when compared with time-matched normothermic controls, n = 4.

In Paper 2, the 8 animals that comprised the study group in Paper 1 were used as controls for the 8 animals that received DA in incrementing dosages during deep hypothermia (25˚C) and during rewarming at moderate hypothermia (30 – 34 ˚C). Randomization of animals between groups was performed simply by blindly assigning every other pig to either group before entering the animal lab.

In Paper 3, 8 animals in the study group were immersion cooled to cardiac arrest, while control animals were cooled by CPB to a pre-determined temperature of 18˚C measured in the oesophagus. After 75 min of deep HCA, animals in both groups were rewarmed by CPB, and observed for 2 h after weaning from CPB. Randomization was performed as in Paper 2.

Fig. 3 shows the protocol flow chart for animals in Papers 1 and 2. In Paper 1, group B in the flow chart is the study group, whereas the 4 control animals are not included. In Paper 2, group A in the flow chart is the study group and group B serve as controls.

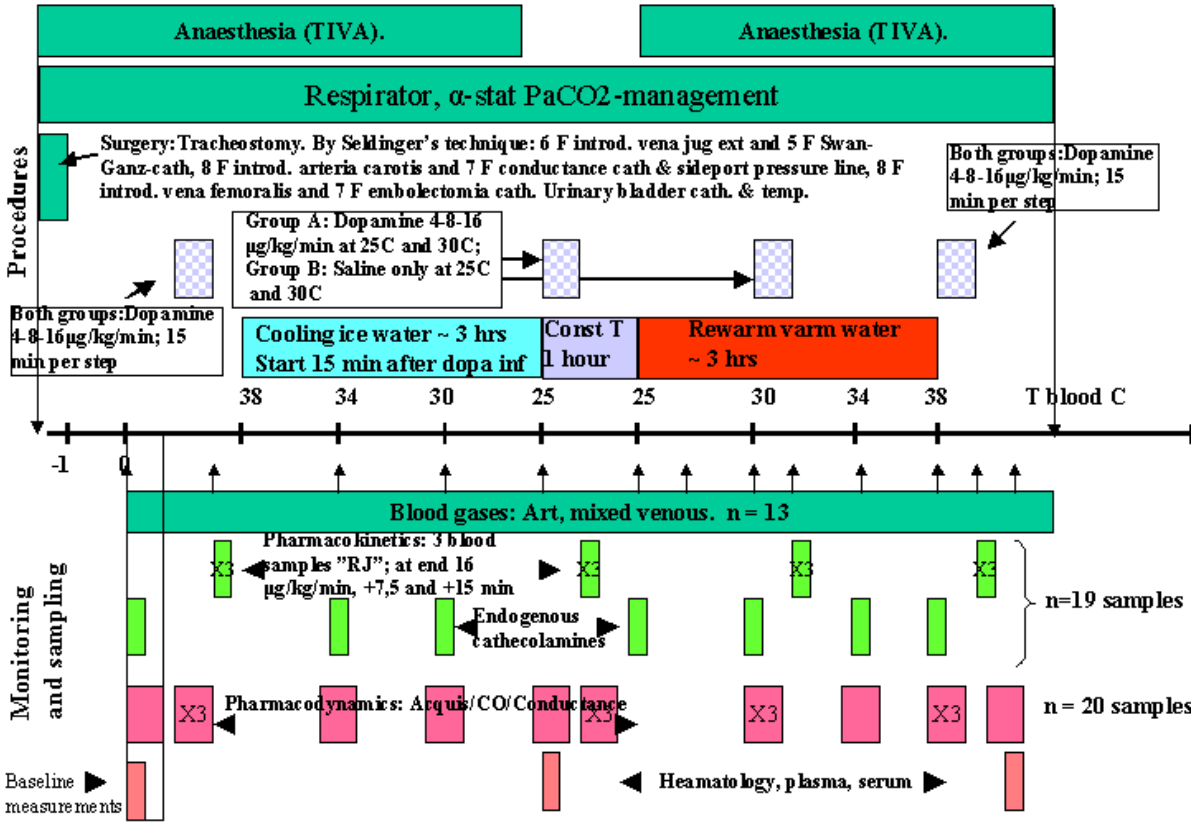

Figure 3. Flow chart for Papers 1 and 2.

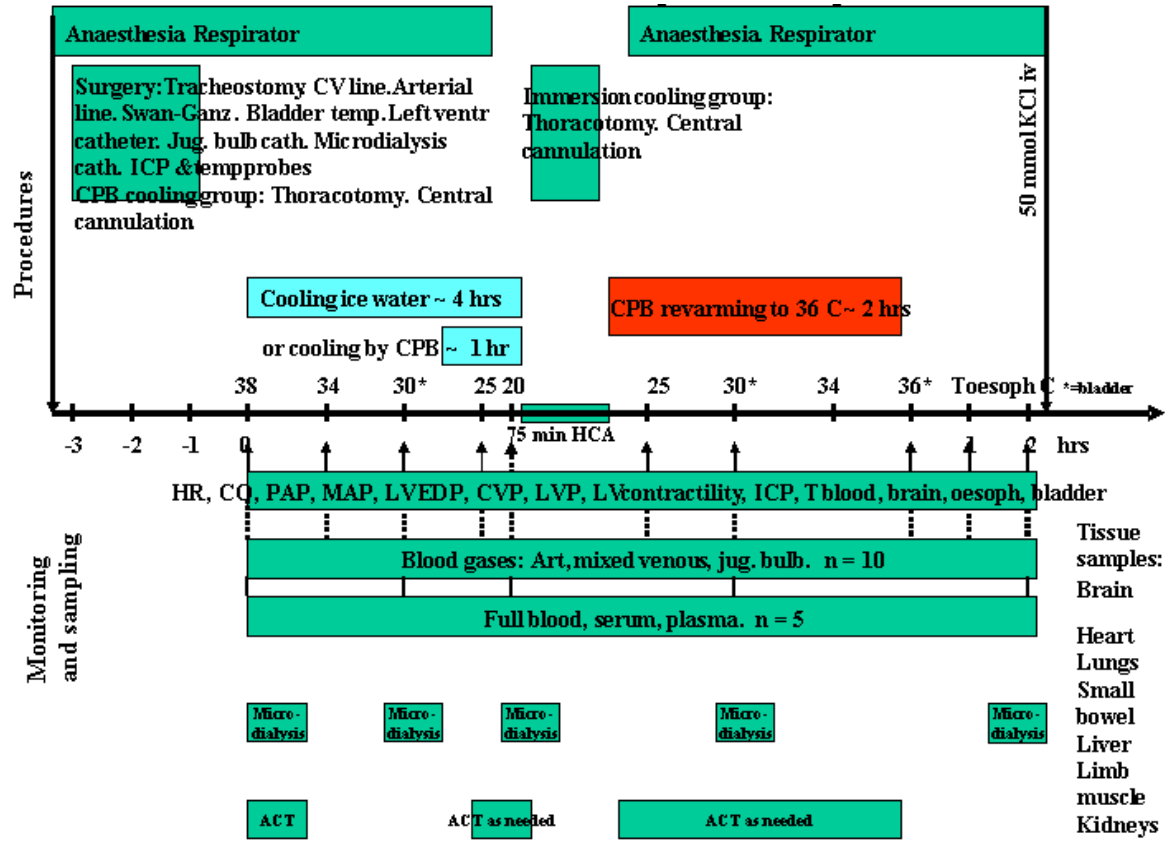

Figure 4. Flow chart for Paper 3.

For clarity, I also include time-course figures for each study, showing the actual measured time-dependent temperatures.

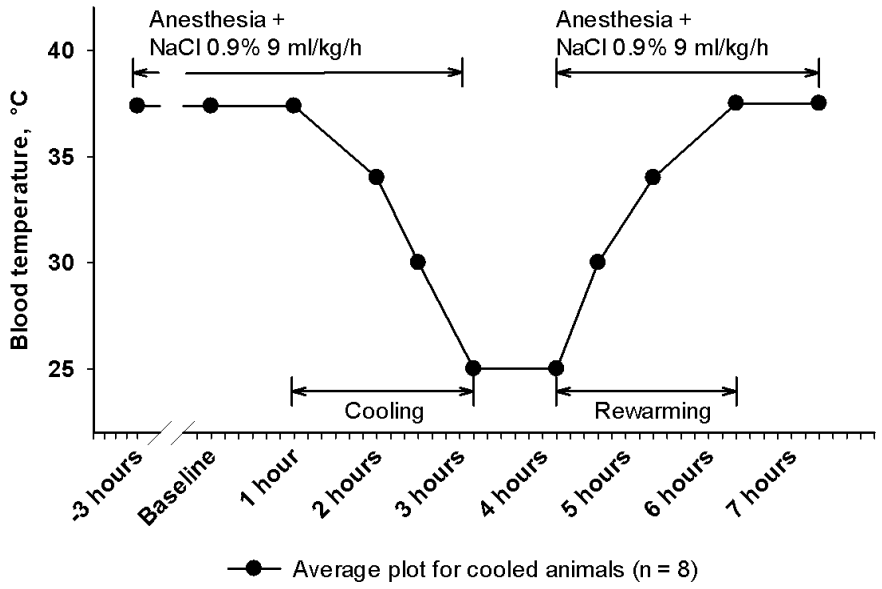

Figure 5. Time-dependent temperatures in Paper 1 (error bars not included).

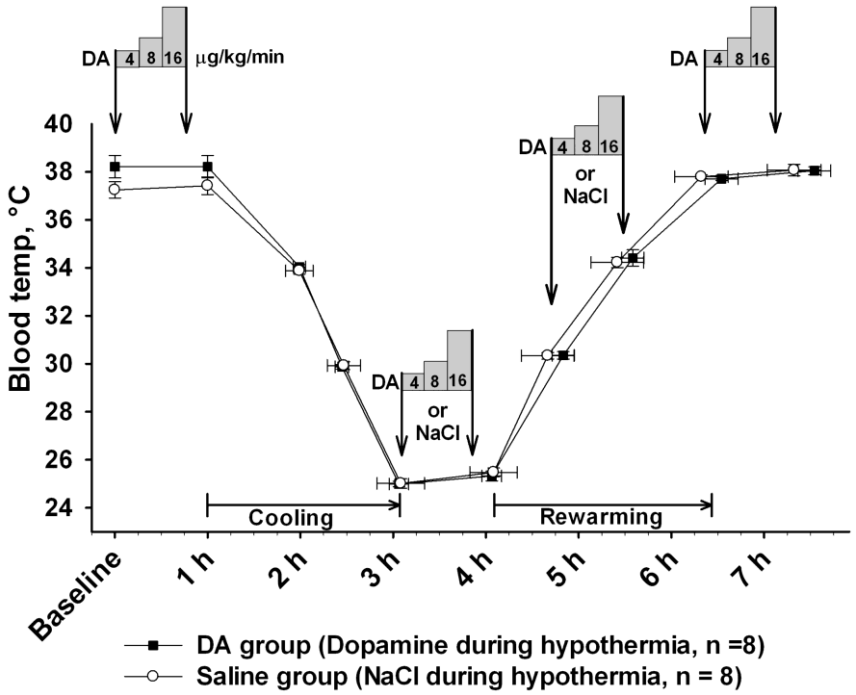

Figure 6. Time-dependent temperatures in Paper 2.

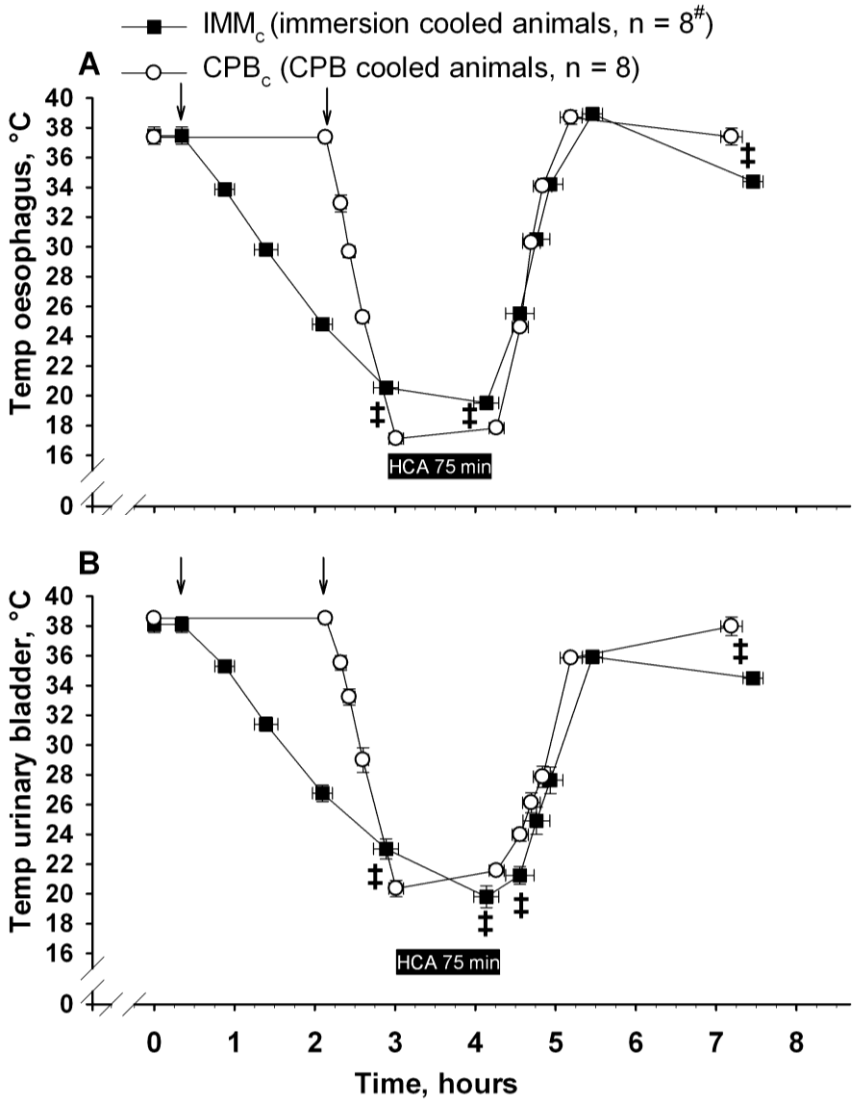

Figure 7. Time-dependent temperatures in Paper 3. *A, Temperatures in oesophagus vs time; B, Temperatures in urinary bladder vs time; HCA, hypothermic circulatory arrest.* ‡ *Significant difference in temperature between immersion cooled and CPB-cooled animals (P ≤ 0.05). Arrows denote start of cooling in respective groups.*

7.4 Anesthesia and respirator protocol

Animals in all series received the same anesthetic protocol. After an overnight fast, anesthesia was induced in the pen by an intramuscular bolus of ketamine hydrochloride 20 mg/kg, midazolam 25 mg and atropine 1.0 mg. After transfer to the animal research operating theatre, a catheter was inserted into an ear vein and a bolus

injection of fentanyl 10 μg/kg and pentobarbital-sodium 10 mg/kg was given. After tracheostomy a continuous right external jugular vein infusion of fentanyl 20 μg/ kg/h, pentobarbital-sodium 4 mg/kg/h and midazolam 0.3 mg/kg/h along with Ringer's acetate 9 ml/kg/h was started and maintained throughout the experiment, except for the one-hour period at 25°C core temperature. After termination of experiments the animals were killed with 20 mmol potassium chloride given as an intravenous bolus. No neuromuscular blockers were used at any time.

Animals were maintained on intermittent positive pressure ventilation and a positive end expiratory pressure (PEEP) of 4 cm H_2O was applied throughout the experiments. FiO_2 was adjusted to maintain PaO_2 >10 kPa, and alveolar ventilation adjusted to keep $PaCO_2$ of 4.5 to 6 kPa uncorrected for temperature (α-stat management).

7.5 Recording and calculation of hemodynamic variables

Recording and calculation of standard hemodynamic variables were done in the same manner in all papers and are only briefly summarized.

ECG from standard leads, heart rate (HR), central venous pressure (CVP), MAP, and pulmonary artery pressure (PAP) were continuously displayed on a data monitor and intermittently recorded using a computer program designed at our department. At pre-determined temperatures CO was measured in triplets, by injecting 5 ml precooled saline in a thermodilution catheter positioned in the pulmonary artery. SV and SVR were calculated as: SV = CO/HR; SVR = (MAP - CVP) · 80/ CO. To index SVR body surface area (BSA) was calculated according to the formula: BSA in m^2 = (734 · body weight · 0.656): 10000 (117). Global delivery and consumption of oxygen (DO2 and VO_2) were calculated as oxygen content in arterial blood · CO, and the difference of arterial and mixed venous oxygen content · CO, respectively.

7.6 Biochemical analyses

7.6.1 Catecholamines (papers 1 and 2)

Blood with heparin (4 IU/ml), reduced glutathione (4.5 mM) and EDTA (5 mM) was kept on ice/water for maximally 30 minutes before plasma was obtained by

centrifugation (1000 × g) for 20 minutes at 4°C. Samples were stored at -80°C awaiting analysis. Plasma samples (1 to 2 ml) were spiked with known concentrations of the internal standard (DHBA = dihydroxy- benzylamine) and added 1 ml 2 M Tris-EDTA buffer (pH 8.7). The catecholamines were adsorbed onto alumina (10 mg). After aspiration of plasma/buffer, the alumina was washed three times with bi-distilled water (1 ml). The catecholamines were eluted from the alumina with a mixture (100 µl) comprising acetic acid (175 mM), sodium bisulfide (9 mM) and EDTA (0.7mM). After whirling and centrifugation, the aquous phase was aspirated and transferred to the autoinjector. DA, norepinephrine and epinephrine were separated by HPLC and their concentrations determined with an electrochemical detector. The analyses were performed at ambient temperature with a flow of 1.2 ml/ml.

7.6.2 Other analyses

Hemoglobin (Hb) measurements, and arterial and mixed venous blood gases uncorrected for temperature were analysed on a blood gas analyser. Blood samples for serum analysis were put on ice, quickly centrifuged and the serum was then quickly frozen and kept at -80°C awaiting analysis. Tumor necrosis factor alpha (TNF-a) was analysed by the quantitative sandwich enzyme immunoassay technique. Troponin T (TnT), ASAT, ALAT and albumin were analysed by the sandwich method of electrochemiluminescence, UV-test with pyridoxal phosphate activation, and a colorimetric end point method.

7.7 Postmortem wet/dry organ weight ratios (Paper 3)

After the animal had died or was killed, autopsy was performed and representative samples of various organs as specified in Fig. 7 were excised, weighed and stored overnight in an incubator at 60°C, before weighing was repeated followed by calculation of wet/dry ratios.

7.8 Conductance catheter methods (Papers 1 and 2)

Since there are changes in circulating blood volume during cooling and rewarming (31;118;119), variations in preload conditions were likely to occur in our experiments.

Because assessing the heart contractility during hypothermia and during catecholamine infusions was one of the main goals in Papers 1 and 2, priority would be given to obtain preload independent measures of contractility.
In an attempt to isolate the intrinsic ability of the myocard to generate force and shortening independent of preload, afterload or heart rate, H. Suga developed the concept of end systolic elastance (Ees), calculated from PV loops during occlusion of the inferior caval vein (120).
The assessment of "true contractility" of the hypothermic heart was our main motif for inserting a conductance catheter (CD Leycom, Zoetermeer, The Netherlands) via the left carotid artery into the left ventricle in animals in Papers 1 and 2.

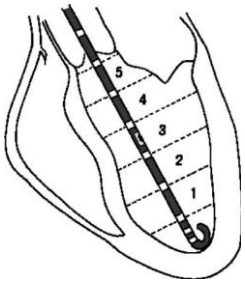

Figure 8. Conductance catheter placement in left ventricle.

The part of the conductance catheter that is placed inside the left ventricle (LV) is equipped with a pressure sensor and with pairs of electrodes; one which create an electrical field wheras the other measure the ensuing conductance which is dependent on the amount of blood surrounding the pair of electrodes. Each pair of measuring electrodes thus measures a slice of blood volume, as is illustrated in Fig. 8. The sum of these blood volumes comprise the volume at any given instant within the left ventricle. Because the electrodes measure relatively few volume slices, the obtained sum of segmental conductances has to be corrected by an independent estimate of SV (121). In our experiments, CO measured by the thermodilution method divided by HR determined the SV against which the sum of segmental conductances was corrected. By correcting conductance-derived SV against this independent method, we also eliminated the effect of blood resistivity on measured conductance.
Several indices of LV contractility and relaxation throughout the heart cycle (Fig. 9)

may be derived from the PV loop. We made use of dP/dt_{max}, dP/dt_{min}, the time constant of isovolumetric relaxation (Tau) based on a monoexponential decay model, end diastolic PV relationship (EDPVR), end systolic elastance (Ees, or end systolic PV relationship (ESPVR)) and preload recruitable stroke work (PRSW). Furthermore, we also included the interaction between LV function and the arterial system, by calculating arterial elastance (Ea) as end systolic pressure/SV and finally calculating arterial-ventricular coupling ratio as Ea/Ees.

The conductance derived LV end diastolic and end systolic volumes (EDV and ESV) resulted from intraventricular conductance and the conductance of surrounding structures (above all the myocardium), called parallel conductance. Parallel conductance determination by use of repetitive hypertonic (30%) saline infusions at each temperature was not performed, as this would have lead to considerable NaCl accumulation throughout the experiment. Consequently, EDV and ESV in our studies did not represent real LV volumes, and no measure of LV ejection fraction could be calculated. However, recording of relative LV volume changes during cooling and rewarming could be performed. PRSW is the relationship between stroke work (see Fig.9) and EDV, and is thus affected by parallel conductance, however it is not the absolute PRSW values that are of interest, but the changes in PRSW due to various interventions. The same reasoning applies to the calculation of EDPVR. The use of PV loops to determine end systolic elastance is not affected by parallel volume determination.

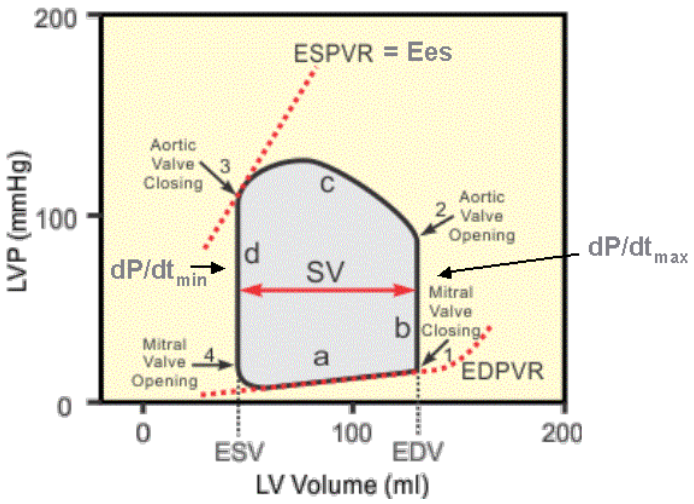

Figure 9. Pressure-volume loop of the heart. *The area within the loop equals the stroke work (SW). a, diastole; b, iso-volumetric contraction; c, systole; d, iso-volumetric relaxation; SV, stroke volume; ESV, end systolic volume; EDV, end diastolic volume; LVP, left ventricular pressure; ESPVR; end systolic pressure-volume relationship; EDPVR, end diastolic pressure-volume relationship; Ees, end systolic elastance, dP/dt_{max}, maximal acceleration of pressure in the cardiac cycle; dP/dt_{min}, maximal deceleration of pressure in the cardiac cycle.*

7.8.1 Determination of end systolic elastance, preload recruitable stroke work and end diastolic pressure/volume relationship

The conductance catheter placement was guided by LV pressure signals being displayed on a monitor and by advancing the catheter to obtain the maximum number of electrode segments displaying ventricular volumes without causing ventricular arrhythmias. Segments lying outside the ventricle were excluded before each recording.

LV contractility indices Ees, EDPVR and PRSW were calculated based on PV recordings during abrupt inferior vena cava occlusions by a balloon catheter advanced via the femoral vein to the inferior caval vein.

Fig. 10 shows the principle for determination of Ees and EDPVR . The slope of the line resulting from the end-systolic points during preload reduction (red dots) denotes Ees, while the slope of the line drawn between corresponding end-diastolic points (blue dots) denotes EDPVR. V_0 was defined by the intercept of the Ees slope of the volume axis (not shown in Fig. 10).

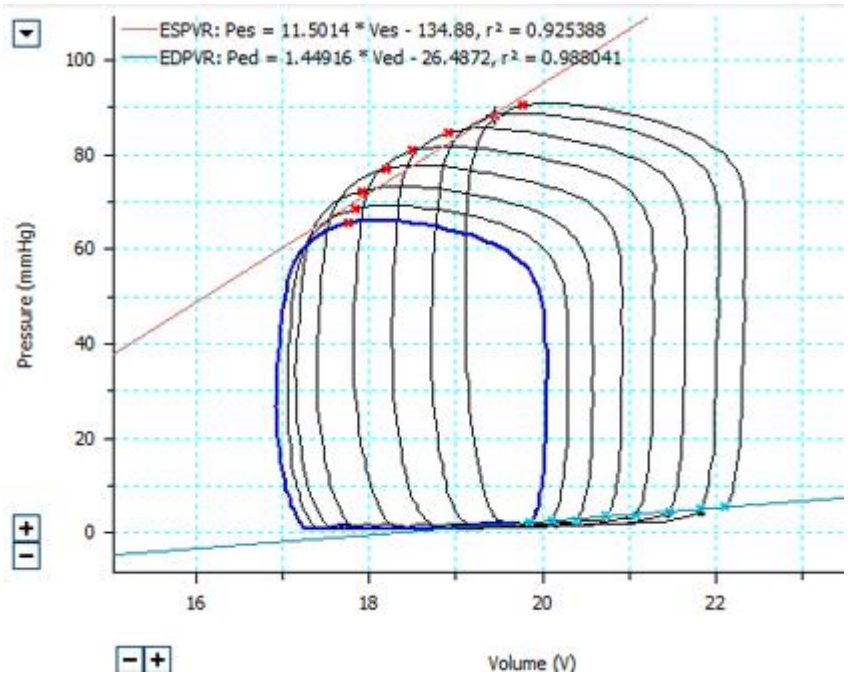

Figure 10. Pressure-volume loops recorded during occlusion of vena cava inferior

Fig.11 shows how PRSW is calculated by plotting SW against EDV during abrupt vena cava occlusions.

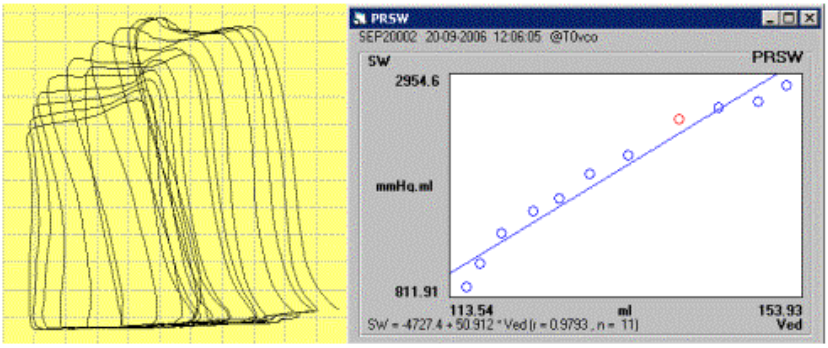

Figure 11. Determination of preload recruitable stroke work (PRSW).

At core temperatures below 30°C inferior caval occlusions did not induce changes in PV loops that could be applied to calculate PRSW, EDPVR or Ees, probably because the heart obtained most of its filling from the superior caval vein in this low-flow state.

7.9 Brain microdialysis and intracerebral monitoring (Paper 3)

7.9.1 Microdialysis

Traditionally, researchers have gained information about cellular and tissue metabolism indirectly via blood samples. During the last two decades, however, microdialysis techniques have been refined to retrieve metabolites from tissue interstitium, making assessment of dynamic changes in isolated organ metabolism more precise (122).

This was why we wanted to apply this method to monitor brain metabolism in Paper 3. An area of approximately 2 x 5 cm of the skull over the right hemisphere was exposed by excision of the scalp. A microdialysis catheter was placed to a depth of 10 mm below the dura mater through a cranial hole 1 cm right to the sagital suture and 2 cm ventral to the coronal suture. The catheter was connected to a 1,0 ml syringe placed into a microinfusion pump and perfused with Ringer solution at a rate of 2,0 µl/min. Sampling time was 30 minutes at five times during the experiment: At baseline, at 30°C and 20°C during cooling, at 30°C during rewarming and 2 hrs after end of rewarming. The microvials containing the dialysate fluid were immediately frozen at -70°C and concentrations of cerebral tissue glucose, lactate, pyruvate, glutamate and glycerol were measured later with a microdialysis analyzer (CMA 600, CMA/Microdialysis).

Fig. 12 shows how exchange of solutes occurs in both directions across a semipermeable membrane of the probe, depending on the orientation of the solute concentration gradients (122).

7.9.2 Other intracerebral monitoring

A pressure-monitoring catheter for recording of intracerebral pressure (ICP) and a temperature probe for monitoring intracerebral temperature was placed in the brain parenchyma just below the dura mater through another cranial hole 1 cm to the right of the sagital suture and 1 cm dorsal to the coronal suture. ICP was displayed continuously on a monitor and recorded manually at different time intervals. CCP was calculated as MAP – ICP. A catheter was placed retrograde through the left internal jugular vein to sample blood for determination of jugular venous oxygen saturation.

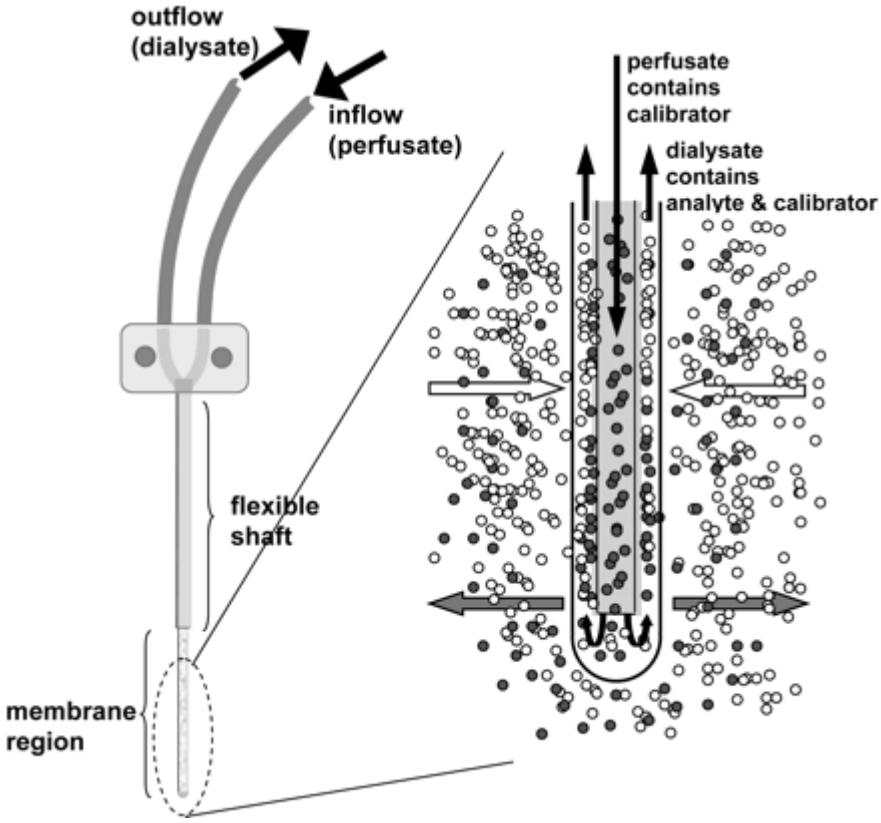

Figure 12. Principles of microdialysis. *A microdialysis probe of concentric design is shown. The magnified membrane region illustrates net diffusion of a compound (analyte) of interest (open circles) into the probe, as well as the net diffusion of the calibrator (closed circles), which has been added to the perfusate, from the probe to the extracellular space* (122).

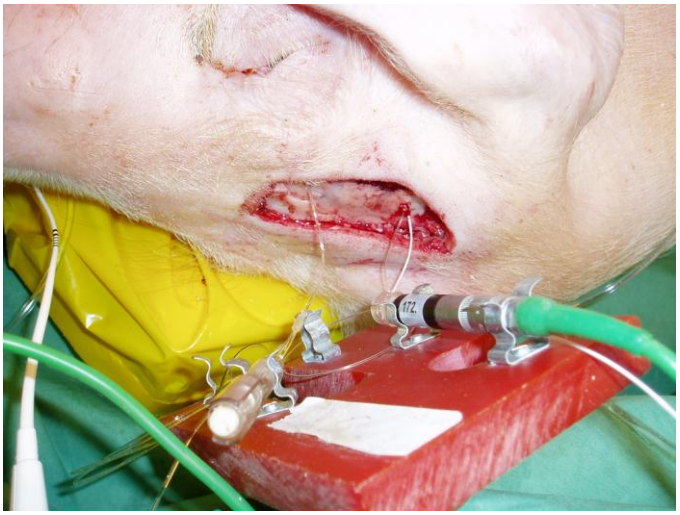

Figure 13. Cerebral monitoring. *Left, microdialysis vial and probe; right, pressure transducer and thermistor*.

7.10 Cooling and rewarming

7.10.1 Temperature monitoring

In Papers 1 and 2 the core temperature was recorded from a thermistor in the thermodilution catheter positioned in the pulmonary artery.

In Paper 3, due to the brain monitoring and the over-all more complex body temperature changes associated with CPB, body temperature was monitored continuously at four places: Brain, oesophagus, urinary bladder (via the urine catheter) and pulmonary artery (via the thermodilution catheter). During CPB, temperatures were also monitored in the venous and arterial lines and displayed on the centrifugal pump machine. Oesophageal temperature was used as reference temperature, determining sampling points throughout experiments and end point of cooling, whereas the point of weaning from CPB during rewarming was determined by urinary bladder temperature. Brain temperature was used to withdraw anesthetics at temperatures below 25°C.

7.10.2 Immersion cooling and rewarming (Papers 1 and 2).

After instrumentation all surgical wounds were sutured in two layers and the animals were laid in a right lateral recumbent position on the operating table. By use of a centrifugal pump and a heat-exchanger, cold water (5°C) circulated the hollow operating table and a tarpaulin tub surrounding the animal. The upper left side of the animal was covered with ice slush and irrigated by cold water leaving two thirds of the dependent animal submerged. The head was placed on a cushion and not immersed or covered with ice slush (Fig. 14). At 26°C core temperature cold water circulation was discontinued, the tub drained for water and ice slush, and core temperature subsequently dropped to approximately 25°C in all animals. To prevent core temperature from a further drop, small amounts of warm water was added to the tub. Rewarming was achieved by circulating the operating table and the tub, and by irrigating the upper left side of the animal, with hot water (40 to 42°C, measured in the afferent water hose) till rewarming (38°C) was accomplished.

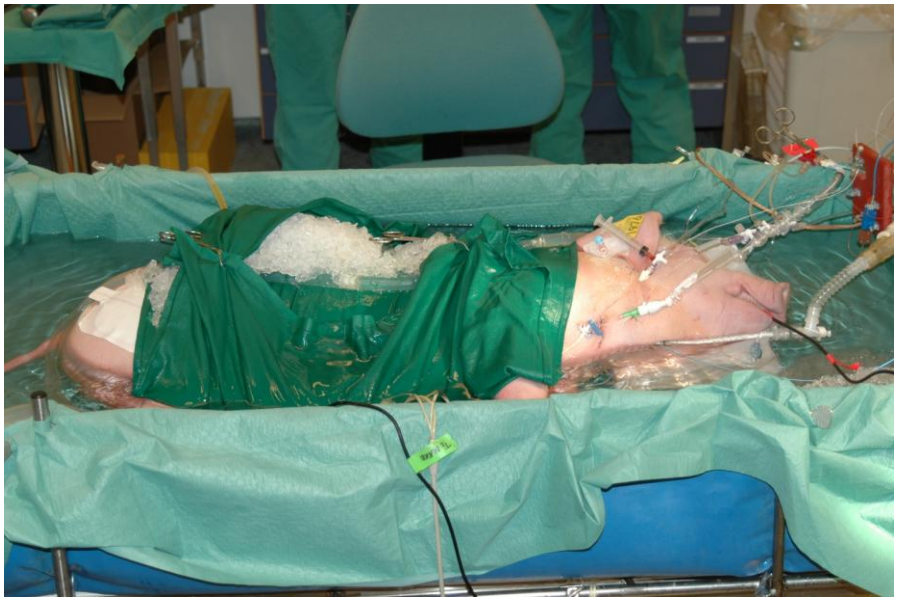

Figure 14. Immersion cooling.

7.10.3 Immersion cooling in Paper 3

Animals in the study group in paper 3 were immersion cooled just like the animals in papers 1 and 2, with the exception that the cooling end point was not a predetermined temperature, but simply HCA. From pilot experiments, we expected asystole to occur at 18 – 22°C. As oesophageal temperature fell below 24°C and serious bradycardia (< 20 beats/min) developed, cold water circulation was discontinued and the tub was drained for water and ice slush. Subsequently oesophageal temperature invariably dropped further, and with the onset of asystole preparations for sternotomy and attachment to CPB started.

7.10.4 Cardiopulmonary bypass (Paper 3)

Animals in the control group were cooled by CPB to a predetermined core temperature at 18°C. Animals in both groups were rewarmed by CPB until 36°C in the urinary bladder.

Extracorporeal rewarming is considered the best rewarming technique in victims of accidental hypothermia that present with asystole or an unsustainable circulation (6). In adult patients, femoro-femoral cannulation is the methode of choice because its simplicity and the possibility to maintain external cardiac compression until bypass is establsihed (123). However, in small children and infants, some clinicians advocate

median sternotomy and central cannulation to improve venous return (124). In our pilot experiments we found the femoral vessels in the juvenile pigs too gracile to insert large enough cannulas to ensure sufficient CPB flow. Therefore we chose central cannulation, allowing us to use a single stage 24 F venous cannula in the right atrium and a 16 F arterial cannula in the ascending aorta.

Other researchers have studied extravasation during experimental hypothermic CPB, utilizing a traditional CPB set-up with a reservoir of stagnant blood that serves as a volume buffer during the bypass (125). The advantage of the reservoir is that volume substitution to the circuit can be titrated so that the fluid level in the reservoir is kept constant (125). The disadvantages are that stagnant blood requires more aggressive anti-coagulation, with the potential of increased bleeding, especially during deep hypothermic CPB, and that circuit priming volume increases, with the result that hemoglobin concentration drops to an un-acceptable low level (< 5 g/dl) in a small animal. Therefore, we chose a heparin-coated circuit without reservoir and with a highly efficient, low-volume oxygenator (Jostra Quadrox, Maquet Cardiopulmonary, Hirrlingen, Germany). Priming volume was thus reduced to about 500 ml and anti-coagulation end point was an activated clotting time (ACT) of around 200 sec.

As appears in Fig. 15, fluid was added to the circuit if a negative access pressure occurred in the venous cannula when pump rotational speed was increased to reach CPB end points of MAP above 50 mm Hg and oxygen saturation in mixed venous blood above 60 %.

7.10.5 Cooling and rewarming by cardiopulmonary bypass

Cooling and rewarming while on CPB was performed using the heat-exchanger attached to the oxygenator to achieve a temperature gradient of maximum 5°C between drain blood in the venous line and inflowing blood in the arterial line. However, during rewarming maximum temperature in the inflowing arterial line was set at 39 °C and the temperature gradient between the urinary bladder and blood in the arterial line was not allowed to exceed 10°C.

When animals in the CPB_c group had been cooled until 18°C in oesophagus, CPB was withdrawn, the aorta was cross-clamped distal to the aortic cannula and 400 ml of cold

(4°C) crystalloid hyperkalemic cardioplegic solution (126) was added via the aortic cannula to produce cardiac arrest.

During rewarming internal electroconversion of VF would be initiated at an oesophagal temperature of 25°C. If three attempts at electroconversion were unsuccessfull up to an oesophagal temperature of 28°C, a bolus of 150 mg amiodarone was administered in the arterial cannula before additional electroconversions.

Weaning from CPB was performed when temperature measured in the urinary bladder reached 36°C and accomplished by use of DA infusions to keep MAP above 60 mmHg.

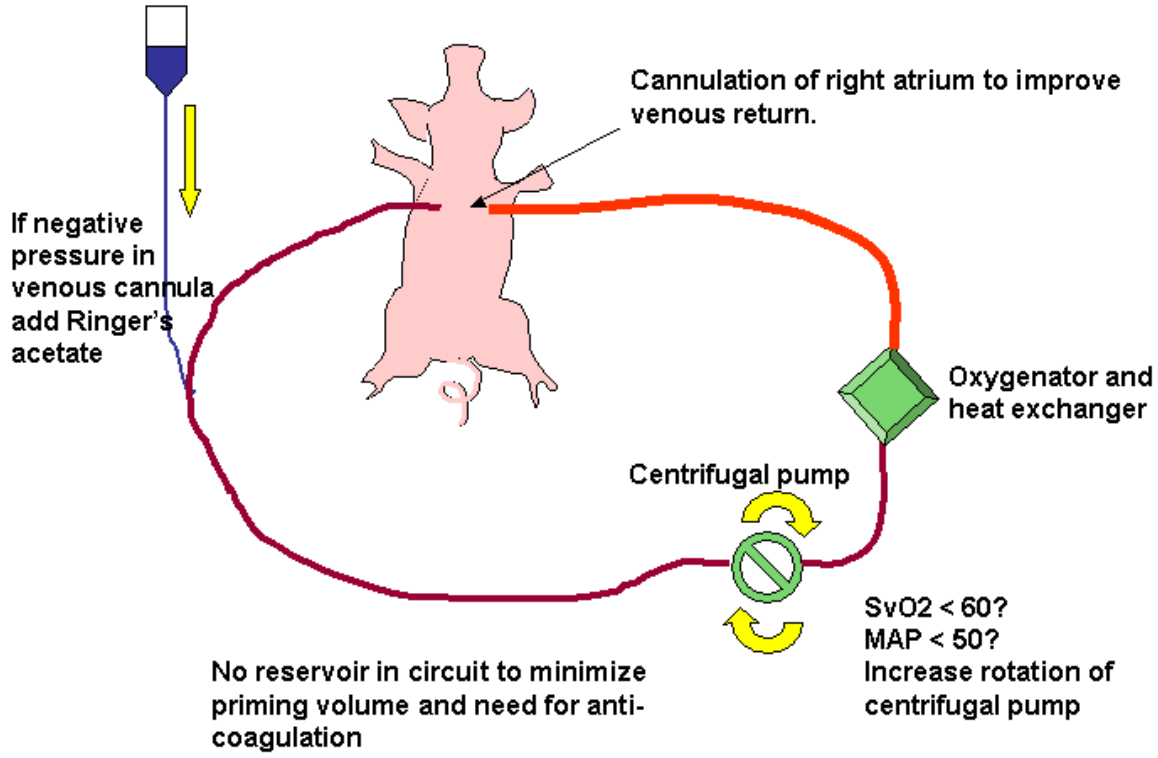

Figure 15. Set-up and end points for cardiopulmonary bypass

7.11 Dopamine as study drug: Characteristics and effects

The reasons for choosing DA as catecholamine study drug in this thesis are the widespread use of DA in perioperative and intensive care, the explicit recommendation for its use in accidental hypothermia guidelines (79) and the above mentioned possible positive effects after testing DA during experimental hypothermic conditions.

DA is an endogenous biogenic amine synthesized from the amino acid tyrosine in nerve axon terminals in the central nervous system, where it acts as a neurotransmitter. As a member of the catecholamine family, DA is a precursor to nor-epinephrine and epinephrine (104).

In man, DA activates DA, and β-adrenergic and α-adrenergic receptors at all dosages, but the relative importance of the effect of receptor activation is dose dependent. Thus in man, low-dose DA (2–4 μg/kg/min) improves renal blood flow and increases diuretic output through activation of DA receptors (105). At moderate-doses DA (4–10 μg/kg/min) in humans, effects from β-adrenergic cardiac receptor stimulation predominate, and CI increases by increasing stroke index (SI) (106). High dose DA (above 10 μg/kg/min) in man engages α-adrenergic receptors relatively more than at lower doses, leading to increased SVR (105).

Despite the vast amount on research of the pharmacology on DA, all but few works address the effects in normothermia, and studies of DA pharmacokinetics in hypothermia are virtually non-existent.

7.12 Statistics

Statistical analyses were performed using SigmaPlot statistical software version 9–11 (Systat Software, Richmond, CA). Intragroup comparisons were performed by one-way repeated-measures analysis of variance, followed by the *post-hoc* test of Holm-Sidak, or if normality test failed, by the test of Student-Newman-Keuls. Paired sample Student's *t* test was used when only single comparisons were made. For comparisons between groups, the two-sample Student's *t* test was used if data showed normal distribution, otherwise Mann- Whitney *U* rank sum test was used. Level of significance was set at $p \leq 0.05$. Data are presented as mean ± SEM.

7.13 Relevance to therapeutic and protective hypothermia

We wanted to develop a model for accidental hypothermia that was as man-like as possible. Cooling would be by immersion in cold water to mimic accidental hypothermia. However, since all animals were deeply sedated, we found our model also to resemble the protocol of surface cooling in therapeutic hypothermia after witnessed cardiac arrest (62).

Originally we studied effects of various dosages of DA at different temperatures within the framework of accidental hypothermia, but it turned out that our findings might also have translational value for DA administration in therapeutic hypothermia.

As one of our aims was to compare physiological responses in the post-hypothermic period following rewarming from surface cooling and cooling by extracorporeal bypass, the model would also have similarities with the clinical practise of applying deep HCA as a protective measure during certain forms of aortic surgery.

8. SUMMARY OF RESULTS

8.1 Paper 1.

Post-hypothermic cardiac left ventricular systolic dysfunction after rewarming in an intact pig model

(all referred figures in this section relate to Paper 1)

8.1.1 Main results

Cooling below 34°C resulted in significantly decreased cardiac contractility that persisted during and after rewarming. In contrast, diastolic function recovered after rewarming. Although mean arterial pressure (MAP) was significantly reduced in hypothermia, SVR increased significantly. After rewarming, SVR was significantly reduced compared with pre-hypothermic baseline. CO was significantly reduced during rewarming, but was maintained at pre-hypothermic levels after rewarming due to a spontaneous increase in HR. The phenomenon of cold diuresis was not encountered since diuresis was unaffected by temperature throughout experiments.

8.1.2 More detailed account of cardiovascular function

i) Mild hypothermia. Ees (Fig. 2B) as the sole indicator of LV contractility, increased significantly at 34°C, as did indexes of diastolic function, Tau (Fig. 3D.) and EDPVR (Fig. 3C). dP/dt_{min} (Fig. 3A) decreased significantly (maximal deceleration decreased). MAP (Fig. 4D) and Ea/Ees (Fig. 3C) decreased significantly at 34°C. All other hemodynamic variables were statistically unaffected by cooling to 34°C.

ii) Immersion cooling below 34°C. Indexes of LV contractility PRSW (Fig. 2A) and dP/dt_{max} (Fig. 2D) decreased significantly during cooling below 34°C, while Ees and Ea/Ees (Fig. 2C) were statistically unaffected. V_0 was statistically indifferent to moderate and severe cooling, as were EDP (Fig. 3B) and EDPVR. During cooling EDV, ESV (Fig. 4F) and CVP decreased, reaching statistical significance at 25°C. dP/dt_{min} was statistically reduced (absolute values decreased) in a linear pattern during cooling, whereas Tau increased in a pattern reciprocal to the dP/dt_{min} curve. CO (Fig. 4A), HR (Fig. 4B), SV (Fig. 4C), and MAP all decreased significantly in a linear way below 34°C. SVRI (Fig. 4E) increased in a nearly similar pattern, reaching

significance at 25°C. Except for cold-induced bradycardia, no arrhythmias were encountered until severe hypothermia was established.

iii) One hour steady state hypothermia at 25°C. No PV data derived from inferior caval occlusions were available at this temperature. Values for dP/dt_{max}, dP/dt_{min}, CO, SV and HR reached their nadir during the 1h period at 25°C, while Tau reached its highest value, as did SVRI.

During stable severe hypothermia sinus bradycardia and various idio-ventricular arrhythmias were seen. The ECG did not show atrial fibrillation nor the so called J wave or Osborn wave, reported as being characteristic of severe hypothermia in humans (127). In 4 out of 8 pigs the phenomenon of mechanical (or pulsus) alternans (128) occurred; that is, a reduced SV observed on the conductance monitor every other heart beat in the absence of corresponding abnormalities in the ECG. One animal got VF that was successfully defibrillated to an organized rhythm during the one-hour period at 25°C.

iiii) Immersion rewarming and the post-hypothermic period. During rewarming PRSW and dP/dt_{max} were significantly lowered when compared to corresponding temperatures during cooling. HR made an abrupt and significant increase during rewarming from 25 to 30°C, and remained significantly elevated at a stable level above this temperature. The other variables that were reduced by cooling tended to approach corresponding values during rewarming in a nearly mirror image pattern.

After rewarming PRSW, dP/dt_{max}, dP/dt_{min} , SV, MAP, EDP and SVRI remained significantly decreased compared to pre-hypothermic baseline values. CVP, EDV and ESV returned to pre-hypothermic controls. While post-hypothermic Ees was statistically unchanged,V_0 was significantly increased after rewarming.

Due to a significant increase in HR, post-hypothermic CO returned to within pre-hypothermic values. Tau and EDPVR, having been increased during rewarming, returned to control. In all animals cardiac rhythm spontaneously returned to sinus rhythm and mechanical alternans disappeared during rewarming.

8.1.3 Other variables

Hemoglobin (Hb) concentration (Fig. 5A) showed a biphasic pattern, decreasing significantly during cooling and increasing significantly during severe hypothermia and rewarming. Oxygen content of arterial and mixed venous blood followed a pattern nearly synchronous with the Hb curve, increasing statistically during severe hypothermia. The global delivery and consumption of oxygen (DO_2 and VO_2, Fig. 5B, C) were reduced by 61 ± 4% and 68 ± 6% respectively during cooling, giving corresponding reductions of 4.7 ± 0.3% and 5.2 ± 0.4% per degree C. While DO2 correlated with temperature in a linear manner, the reduction in VO_2 was just about 1% per degree C in the temperature interval between 38 and 34°C and about 7.8% per degree C from 34 to 25°C, reflecting that the relationship between VO_2 and decrease in temperature took the form of a negative exponential function.

During rewarming DO_2 returned to pre-hypothermic baseline values following a mirror image of the pattern during cooling, while VO_2 normalized in a more linear way than during cooling. Albumin values (Fig. 6A) decreased significantly during cooling and remained significantly reduced after rewarming. Significant increases in both troponin T (TnT, Fig. 6B) and TNF-a (Fig. 6C) serum concentrations were seen after rewarming, both in contrast to their own baseline values and to time matched controls. Serum concentrations of DA, epinephrine and nor-epinephrine were statistically unchanged from pre-hypothermic values throughout the experiments. Diuretic output was not affected by temperature throughout experiments.

8.1.4 Immersion cooling and rewarming rates; temperatures and shivering

Immersion cooling to 25°C lasted 125 ± 15 minutes giving a cooling rate of 6.8 ± 0.7°C/h. Rewarming lasted 133 ± 6 minutes, giving a rewarming rate of 6.0 ± 0.3°C/h. Visible shivering took place in all pigs at the start of cooling, but subsided with progressive cooling and was absent at 25°C. Little, if any, shivering was observed during and after rewarming.

8.2 Paper 2

Changes in cardiovascular effects of dopamine in response to graded hypothermia *in vivo*

(all referred figures and tables in this section relate to Paper 2)

8.2.1 Main results

DA half life (T1/2) was 5.4 ± 0.7 min at normothermia, increased to 11.6 ± 0.8 min at 25°C, but returned to control during rewarming at 34–35°C.

DA infusion at 25°C elevated DA plasma concentration four-fold compared to the same infusion rate at normothermia, leading to increased SVR not seen at normothermia. Also, in contrast to the DA-mediated increase in cardiac index observed at normothermia, high dose DA at 25°C left cardiac index unchanged despite a concomitant increase in HR, since stroke index SI decreased by 43%. During rewarming, cardiovascular effects of DA at moderate hypothermia (30 –34°C) were principally similar to responses during normothermia. DA administration during hypothermia did not blunt post-hypothermic effects of DA, nor did DA administration during rewarming increase the rate of rewarming.

8.2.2 More detailed account of cardiovascular effects

i) Normothermia. The dose-response relationship of DA was the same in both groups at normothermia. Indexes of LV contractility dP/dt_{max} (Fig. 2A) and PRSW (Fig. 2B) increased significantly with incrementing DA dosage steps. As for DA effects on diastolic function, dP/dt_{min} increased significantly at the highest DA step (Fig. 3A), and Tau (Fig. 3B) decreased significantly at each DA dosage increment. Both systolic and diastolic LV volumes (Figs. 2C and 3D), along with EDP, (Fig.3C) were significantly reduced at the 16 μg/kg/min DA dosage step. CI (Fig. 4A) increased significantly at the mid- and high-DA dosage steps due to a synchronous increase in HR (Fig. 4B), since SI (Fig. 4C) was mainly unaffected by DA. MAP (Fig. 4D) was unaffected by DA, while SVR index (SVRI) (Fig. 4E) decreased significantly at each DA dosage increment. CVP (Fig. 4F) decreased significantly with incrementing DA steps.

ii) Severe hypothermia (25°C). LV dP/dt_{max} increased significantly in response to DA infusions of 8 and 16 μg/kg/min, while dP/dt_{min} and Tau were influenced by DA

identically to during the normothermic condition. Ventricular volumes, EDP and CVP decreased significantly at the mid- and high-DA dosage steps at 25°C. Since caval vein occlusions at 25°C caused no changes in PV configuration, no data for PRSW could be recorded at this temperature. As during normothermia, low-dose DA (4 μg/kg/min) reduced SVRI significantly at 25°C. However, unlike during normothermia, the high-dose DA (16 μg/kg/ min) infused at 25°C increased SVRI significantly, and both SVRI and MAP were significantly elevated in the DA group compared with the saline-treated animals.

DA increased HR relatively more at 25°C than at normothermia (59% vs.30% in response to DA 16 μg/kg/min). But as a consequence of the significant lowering of SI during DA infusions (8 and 16 μg/kg/min), DA had no effect on CI at 25°C. Abrupt termination of the highdose DA infusion at 25°C caused significant reductions of SI and CI (data not shown).

iii) Rewarming from 30°C to 34°C. Changes in cardiovascular variables from baseline at 30°C were brought about by the combination of DA infusions and rewarming in the DA group, while changes in the saline group resulted from rewarming solely. Therefore, only significant intergroup differences at the same time points after start of infusions could be attributed to DA effects during rewarming from 30°C. DA brought about significant increases in PRSW at all dosage steps, and in dP/dt_{max} at the medium and high dosage step when compared to control animals. Both EDV and ESV were significantly reduced by DA infusion at 16 μg/kg/min whereas dP/dtmin, Tau, and EDP were unaffected by DA during rewarming. While CI and HR increased significantly at the highest DA dosage step, SI, MAP, and SVRI were unaffected by DA during rewarming.

iiii) After rewarming. There were no intergroup differences in any hemodynamic variable after rewarming to 38°C, except for a significantly increased CVP in animals treated with DA during hypothermia. There were no differences between groups in responses to DA after rewarming, which in principle were identical to the prehypothermic response.

8.2.3 Influence of dopamine on immersion cooling and rewarming rates

While overall cooling and rewarming rates did not differ significantly between the two groups (Fig. 1), DA-treated animals rewarmed more slowly (0.11 ± 0.01°C/min) from 25°C to 30°C than did saline-treated animals (0.15 ± 0.01°C/min) ($p < 0.05$). DA infusions did not significantly accelerate rewarming rates between 30 and 34°C.

8.2.4 Effects of dopamine on oxygen variables at different temperatures

DA caused a significant increase in hemoglobin beyond the hypothermia-induced hemoconcentration, both in severe hypothermia and during rewarming from 30°C (Fig. 5A). Global DO_2 (Fig. 5B) changed in synchrony with CI, hence DA increased DO_2 in normothermia but had no effect on DO_2 at severe hypothermia. Also, 15 min after DA infusion was terminated at 25°C, DO_2 was significantly reduced due to reduced CI compared to saline controls. However, an increase in oxygen extraction from hemoglobin (Fig. 5D) compensated for the reduced DO_2, leaving global oxygen consumption similar in both groups throughout the experiment (Fig. 5C).

8.2.5 Effect of dopamine on diuresis at various temperatures

Total diuresis throughout experiments as well as average diuretic output per hour did not differ significantly between groups. Diuretic output between measuring points was statistically unaffected by either temperature or DA in any group throughout experiments.

8.2.6 Pharmacokinetics of dopamine: Plasma concentrations and half life

At severe hypothermia, DA infusion yielded plasma concentrations about four times higher than at the same infusion rate in normothermia (Table 1). DA T1⁄2 was more than doubled at this temperature (from 5.5 ± 0.7 min at 38°C to 11.7 ± 0.8 min at 25°C) (Fig. 6). During rewarming from 30°C, as temperature approached 34–35°C, DA plasma concentration and T1⁄2 were normalized. Animals in the DA group had some DA remnants at the start of DA infusions at

25°C, 30°C, and 38°C (after rewarming), amounting to < 5% of the preceding concentration measured (Table 1), but without detectable hemodynamic effects.

8.3 Paper 3.

Cooling to hypothermic circulatory arrest by immersion cooling vs. cardiopulmonary bypass: Worse outcome in immersion cooled pigs

(all referred figures and tables in this section relate to Paper 3)

8.3.1 Main results

Survival rates 2h after completed rewarming were 4 out of 8 immersion- cooled animals (IMM_c group) and 8 out of 8 CPB-cooled animals (CPB_c group). Compared with animals in the CPB_c group, animals in the IMM_c group had significant reductions in CO, global delivery of oxygen, MAP and cerebral perfusion pressure during cooling below 25ºC as well as after weaning from CPB after rewarming. Post-hypothermic brain microdialysate data showed significant increased lactate/pyruvate ratio in immersion cooled animals compared to animals in the CPB_c group.

8.3.2 *More detailed account of flow, pressures and oxygen variables*

i) Cooling. Except from the initial reduction in global blood flow delivered by the extracorporeal circuit compared with the flow generated by spontaneous circulation, animals in CPB_c group had a significantly higher blood flow below 34ºC than IMM_c animals (Fig. 3A). However, as Hb content was significantly lower in the CPB_c group after initiating CPB, and throughout cooling (Fig. 4A), DO_2 (Fig. 4D) was significantly higher in IMM_c animals until 25ºC. Below 25ºC CPB_c animals had significantly higher DO_2 than IMM_c animals. Global extraction of oxygen, measured by SvO_2 (Fig. 4 B), matched variations in DO_2 so that VO_2 (Fig. 4E) was independent of DO_2. Brain oxygen extraction, measured by $SvjO_2$, followed the pattern of the SvO_2 graphs, but the difference between groups was more profound for $SvjO_2$ than for SvO_2 at 20ºC (Fig. 4C). MAP (Fig. 3B) declined steadily in IMM_c group while SVR (Fig. 3C) increased throughout cooling. In CPB_c group MAP stabilized after an initial drop at onset of CPB, while SVR was unaffected by cooling. Consequently, at 20ºC

MAP was significabtly lower in the IMM_c group than in the CPB_c group, while the opposite was the case for SVR.

During cooling ICP declined in IMM_c group while it was stable in CPB_c group, resulting in significantly higher values in the CPB_c group at severe hypothermia (Fig. 3D). However, with the decline in MAP in the IMM_c group reported above, CPP in this group was significantly lower than in the CPB_c group at 20ºC (Fig. 3E).

ii) Rewarming. Except from initially increased SVR and Hb in IMM_c group directly after start of rewarming by CPB, there were no intergroup differences in CPB flow, MAP, SVR, ICP, CPP or oxygen variables during rewarming. However, after rewarming was completed, just before weaning from CPB, CPP and SvjO were significantly higher in the CPB_c group.

iii) Post-hypothermic period. 4 of 8 animals in IMM_c group died at the time of weaning from CPB or shortly thereafter due to apparent electro-mechanic dissociation as the heart had no measurable output in spite of electrical activity. In contrast, all animals in CPB_c group survived 2h of post CPB. In order to diminish loss of statistical power, comparisons of variables between groups after rewarming have been made at the time-point directly after weaning from CPB, when five animals in IMM_c group were still alive.

Animals in the IMM_c group had significantly lower CO, HR, MAP, CPP, SvO_2, $SvjO_2$ and DO_2 after weaning from CPB compared with CPB-cooled animals (Table 1). SV was significantly reduced from pre-hypothermic baseline within groups, but there was no inter-group post-hypothermic difference, indicating that lack of chronotropic response in the IMM_c group caused the reduced post-hypothermic CO in this group. dP/dt_{max} and dP/dt_{min} were significantly reduced within IMM_c group from pre-hypothermic baseline, but post-hypothermic intergroup differences did not reach statistical significance. CVP and average DA dose to support the circulation (7.4 ± 1.6 µg/kg/min in IMM_c group vs 2.1 ± 1.2 µg/kg/min in CPB_c group) were both significantly higher in IMM_c animals (Table 1).

8.3.3 Brain metabolism during cooling and rewarming

Brain lactate and glycerol were significantly increased in both groups at the end of experiment, while brain glucose/lactate ratio was decreased (fig 5). At end of experiment brain pyruvate was significantly lower in IMM_c than in CPB_c group, while brain lactate/pyruvate ratio was significantly depressed in IMM_c group compared to in CPB_c animals.

8.3.4 Biochemical variables

Plasma albumin values decreased significantly in CPB_c group after initiating CPB during cooling, both compared to their baseline values and against values in IMM_c animals (Fig. 6). However, during and after rewarming the relation between groups was reversed; significantly higher albumin values in CPB_c group at end of experiment. This was the only significant intergroup difference in biochemical variables at this time-point.

8.3.5 Fluid administration rates, weight gains and organ wet/dry ratios

Fig.7 A demonstrates that fluid volume administration rate was significantly higher during cooling by CPB than by immersion cooling. However, fluid administration rates in immersion cooled animals were significantly higher than animals in CPB_c group during rewarming by CPB. However, no differences between groups in overall fluid administration rates and total body weight gain ratios were found. Post-mortem wet/dry organ weight ratios (Fig. 7B) showed statistically significant increased water content in heart and gut in IMM_c group compared to CPB_c group.

8.3.6 Cooling/rewarming times and temperature measurements

Fig. 2 shows that total experiment times did not differ significantly between groups (10h, 28 ± 7 min in IMM_c and 10h, 11 ± 8 min in CPB_c group). As expected, cooling was significantly slower in IMM_c group (1 h 52 ± 10 min) compared to in CPB_c group (52.8 ± 2.8 min, $p < 0.001$). But as considerable time was used for surgical preparation in the latter group, there was no significant time difference between groups in reaching cooling end-points, measured from baseline. Rewarming lasted significantly longer in

the IMM_c group (1h 19 ± 7 min) compared to in the CPB_c group (56 ± 3 min, p = 0.007). As expected total CPB time was longer in the CPB_c group (1 h 48 ± 5 min) compared to 1h 19 ± 7 min rewarming time in the IMM_c group (p = 0.005). Noteworthy, surviving IMM_c animals did not manage to preserve body heat after weaning from CPB, resulting in significantly lower temperatures than in the CPB_c group at end of experiments (Fig 2 A and B).

9. DISCUSSION

9.1 How to assess systolic and diastolic function in hypothermia?

The view that Ees is an applicable contractility index in various pathological states affecting myocardial function has been challenged by Aghajani et al (116). In experimental normothermic heart failure, they demonstrated significant increases in Ees in stunning, acute myocardial ischemia and endotoxemia, despite significant reduction in other LV functional variables (CO, dP/dt_{max} and MAP) (116). The increase in Ees was accompanied by significant increase in V_0, which resulted in apparently steeper Ees slopes (116).

In Paper 1, Ees was unaffected by hypothermia at 30˚C, and showed no reduction after rewarming, as opposed to other indices of systolic function (PRSW, dP/dt_{max} SV and MAP) (62). Also, in concordance with the findings in experimental heart failure by Aghajani et al (116), we demonstrated a post-hypothermic significant increase in V_0, possibly resulting in a "falsely" increased ESPVR slope (62). We therefore concluded that Ees was an unreliable contractility index in hypothermia.

Instead, in Paper 2 we resorted to the related index, PRSW, which had been used to determine LV cardiac contractility during earlier experiments of cooling and rewarming and found to be a more robust index of contractility than Ees during hypothermia (70). As PRSW results from SW divided by EDV, it incorporates both systolic and diastolic function, and therefore serves as an integrated index of global ventricular function. Therefore, it may not be the ideal index to separate systolic from diastolic function, as was our aim in Paper 1. Furthermore, as pointed out in the *Results* section, PRSW (and Ees) was of no use in severe hypothermia, as VCO had no effect on the PV loop configuration.

It seems that VCO-based contractility indexes alone are of limited value in experimental hypothermia research below 30˚C. One could argue that, in the absence of VCO-derived contractility data at 25°C, single beat estimates of contractility could have replaced traditional deloading variables. However, in vivo single beat contractility estimations in pig have been shown to be unreliable and no better than dP/dt_{max} in predicting LV contractility (129).

Therefore, information from "conventional" indices of myocardial contractility (SV, MAP) and non-VCO-related indices derived from the conductance catheter (dP/dt_{max} and ESV) may be synthesized to fill in the gaps of information on systolic function in severe hypothermia. Likewise, even if data on EDPVR may not be obtained, the indices dP/dt_{min}, Tau, LVEDP and EDV give information on diastolic function even in deep/severe hypothermia.

9.2 Systolic, but not diastolic cardiac failure after rewarming from 25°C

In Paper 1 we concluded that the post-hypothermic cardiac state is characterized by systolic failure with maintained diastolic function. This clearly makes post-hypothermic cardiac dysfunction different from myocardial stunning, where not only systolic, but also diastolic dysfunction is an invariable finding (130).

Hypothermia-induced cardiac failure shares similarities with myocardial stunning in that both conditions involve intracellular Ca^{2+} overload which may partly be attributed to hypothermia-induced inhibition of the Na+/K+-ATPase in the sarcolemma, partly to impaired clearance of free cytosolic Ca^{2+}. While the proposed mechanism in hypothermia is a temperature-dependent attenuation of ion transport mechanisms (73), the concept of myocardial stunning is invariably linked to the ischemia-reperfusion syndrome where dysfunction of ion transport mechanisms due to lack of ATP, together with production of reactive oxygen species, causes Ca^{2+} overload and alteration of contractile protein function (131). The post-hypothermic increase in plasma TnT in Paper 1, although minute in a clinical context, probably was due to Ca^{2+} mediated activation of intracellular proteases that degrade troponins in the same manner as has been suggested to take place during myocardial stunning (131).

During hypothermia changes in diastolic functional variables were observed. Most prominent was the increase in isovolumetric relaxation, Tau, and decrease in dP/dt_{min}, indicating a temperature-dependent slowing of sarcoplasmic reticulum (SR) function that together with potentially elevated intracellular Ca^{2+}concentration delayed clearance of cytosolic Ca^{2+}. This finding corresponds well with the fact that diastolic Ca^{2+} extrusion depends mainly on SR Ca^{2+} pump activity, also during hypothermia

(132), and that enzyme kinetics of the Ca^{2+} pump is highly temperature dependent (Q_{10} effect). The passive, late diastolic phase, the filling phase, was less compromised during hypothermia as indicated by a modest increase in EDPVR in the present experiment.

The effects of change in temperature on the SR Ca^{2+} pump are demonstrated by the return to control of Tau after rewarming. The post-hypothermic decrease of dP/dt_{min} may imply an early diastolic dysfunction which remained from the hypothermic period. However, Tau and EDPVR returned to pre-hypothermic levels, EDV was statistically unchanged and EDP was even reduced after rewarming. We interpret the post-hypothermic decrease in dP/dt_{min} as resulting from the concomitantly decreased MAP, and suggest that posthypothermic early, as well as late diastolic function is normalized after rewarming. This is in accordance with previous findings that diastolic function was restored in post-hypothermic core cooled dogs (70).

To summarize, we propose that elevated intracellular Ca^{2+} concentration during hypothermia did cause post-hypothermic systolic failure through modification of contractile proteins. However, there was never a lack of ATP causing sustained diastolic stiffness, an aspect known of myocardial stunning. Diastolic Ca^{2+} extrusion was restored to pre-hypothermic levels in synchrony with the restoration of the highly temperature dependent enzyme kinetics of the Ca^{2+} pump.

9.3 Post-hypothermic interaction between heart and vasculature

There was no sign that the modest post-hypothermic reductions of MAP and SVR after immersion cooling to 25˚C affected organ blood perfusion, and since CO was maintained, the phenomenon of rewarming shock was not encountered. Our protocol of iv administration of Ringer's acetate at 9 ml/kg/h probably prevented hypovolemia during and after rewarming. In stead, post-hypothermic reductions of MAP and SVR could be interpreted as an adaptive measure by the organism rather than as a sign of incapacitation of vascular tonus control. As mentioned in chapter 5.4.6 in the *INTRODUCTION* section, the interaction between vascular tone and cardiac contractility is expressed through the Ea/Ees coupling ratio (60;61). Ea in itself, being the ratio between LVESP/SV, is not a variable solely derived from the vascular

system, and as such does encompass more than SVR. Effective coupling of heart to artery may be defined as the optimal transfer of blood from heart to periphery without excessive changes in blood pressure, and it has been reported that an Ea/Ees coupling ratio of 0.6 to 1.2 represents a near optimal relation between work and efficiency (61). When systolic heart failure (low Ees) is accompanied by high arterial elastance (high Ea), the Ea/Ees is elevated and the unfavorable situation of afterload mismatch occurs (61). In the present study post-hypothermic Ea/Ees was statistically unchanged from baseline. If MAP and SVR had remained unchanged from pre-hypothermic values, Ea would have been increased, possibly leading to an Ea/Ees ratio significantly increased in an unfavorable direction. In our opinion the reduced post-hypothermic SVR, in the presence of reduced LV contractility and SV, may imply an optimized arterial-ventricular coupling ratio that together with increased HR normalizes CO and DO_2.

9. 4 Cooling below 25˚C: When does spontaneous circulation become unsustainable?

The post-hypothermic cardiac failure after rewarming following 1h hypothermia at 25ºC in Paper1 was mild and well compensated. While Paper 1 relates to global variables, previous experiments in our group using dogs cooled to 25°C demonstrated the absence of myocardial oxygen or substrate deficits (70). It seems therefore that experimental cooling to 25°C is relatively well tolerated. However, in Paper 3, immersion cooled animals (IMM_c group), in contrast to animals cooled by CPB (CPBc group), were exposed to a protracted period during cooling below 25°C, lasting from 30 to 65 min, with continuously decreasing organ perfusion, evident as marked reduction in CO and MAP. Despite a higher Hb concentration in the IMM_c group than in CPBc animals whose blood was diluted by the extracorporeal circuit, DO_2 fell as a result of the lower CO in the IMM_c group. Due to increased extraction of oxygen from Hb, apparent from a reduced SvO_2, immersion cooled animals managed to keep global oxygen consumption at the same level as pigs that were maintained on CPB. However, it has been demonstrated that a MAP of 40 mmHg during hypothermic CPB may lead to cerebral ischemia, indicated, among other variables, by increased levels of lactate and lactate/pyruvate ratio in microdialysate fluid (133). Our finding of significantly

increased brain lactate in IMM_c animals compared with CPB_c animals at onset of HCA was preceded by significant drops in both MAP, CPP and SvjO2. The more than doubled SVR in IMM_c animals compared with CPB_c animals below 25°C may have contributed to a compromised tissue perfusion. Taken together, a core temperature of 25°C in the pigs used in Paper 3 seems to represent a "breaking point", below which spontaneous circulation was no longer sustainable for all organ systems, even if global VO_2 was unaffected. This parallels to observations in rats maintained at 15°C (which is above the temperature level where asystole is reported to occur in rat hearts) for 1 – 4 hours (72). Although global oxygen supply was not a limiting factor during the ensuing rewarming (72), there was a hypothermia-induced myocardial ATP depletion and lactate accumulation after rewarming that could be comparable with the characteristics of low-flow ischemia (47).

The macroscopic phenomenon of "stone heart", or irreversible ischemic contracture, occurred in 4 of 8 immersion cooled animals in Paper 3 during the rewarming. Before cardioplegic solutions and hypothermic bypass were routinely undertaken during the 1970's, this clinical phenomenon, later linked to cytosolic Ca^{2+} accumulation (134), was a feared complication to ischemic cardiac arrest during heart surgery (135). The fact that "stone hearts" were not encountered during rewarming in the CPB_c group, which also had undergone a period of 75 min of deep HCA, emphasizes the detrimental effects of long-lasting severe hypothermia in combination with unsustainable perfusion in terminal immersion cooling.

While animals that were immersion cooled to 25˚C in Paper 1, as well as animals that were cooled by CPB in Paper 3, may have adjusted to a mild post-hypothermic cardiac failure by increasing HR and lowering SVR while maintaining an adequate MAP, there were seemingly no such mechanisms present in surviving immersion cooled animals in Paper 3 after rewarming. Despite a CO that was reduced by nearly 50% compared with baseline, there was no compensatory increase HR, which was at pre-hypothermic baseline level. Furthermore, post-hypothermic SVR was also unchanged from pre-hypothermic baseline, offering no relief for the failing heart. The apparent loss of post-hypothermic systemic cardiovascular autoregulation in animals that were immersion cooled to HCA raises the question whether neuronal control centres in

the brain stem had been damaged as a consequence of immersion cooling below 25 ˚C.

9.5 Pharmacokinetics of dopamine in hypothermia

Our finding that DA T½ was more than doubled at 25°C indicates a reduced clearance, but the assumed plasma reduction that is discussed further in section 9.6.2. probably had an additional effect due to a decrease in the apparent volume of distribution.

Assuming first-order elimination kinetics, a DA infusion rate of about 4 μg/kg/min at 25°C could be comparable to 16 μg/kg/min in normothermia, given the fourfold increase in DA plasma concentration at 25°C. During rewarming, at the temperature goal zone for therapeutic hypothermia, DA plasma concentration and T½ normalized, illuminating the difference in pharmacokinetics between severe and moderate hypothermia.

There is the possibility that low temperature change kinetics from first order to zero order. However, our data fit nicely to a first order elimination curve, an observation that suggests the existence of first order elimination.

9.6 Effects of dopamine in the normothermic and hypothermic pig

9.6.1 Normothermia

Despite similarities between man and pig in cardiovascular physiology, the present study disclosed differences between these species in cardiovascular effects of DA. In man, low-dose DA (2–4 μg/kg/min) improves renal blood flow and increases diuretic output through activation of DA receptors (105). This diuretic response was absent in our piglets, a finding also reported by other researchers (136;137). Conversely to what is observed in man DA did not affect SI in our pigs, but CI increased as a result of increased HR, a finding which is supported by others (136). While high dose DA (above 10 μg/kg/min) in man engages α-adrenergic receptors relatively more than at lower doses, leading to increased SVR (41), incrementing DA doses in normothermia resulted in decreasing SVR in the present study, as reported also by others (136).

9.6.2 Moderate hypothermia

Earlier experimental studies have demonstrated that rewarming from severe hypothermia reverses hypothermia-induced plasma reduction (discussed below) (31;32;119). As DA pharmacokinetics normalized, it could be expected that the response to DA would resemble the normothermic state with increased HR, maintained SI, and consequently increased CI as temperature approached 34°C in Paper 2.

9.6.3 Dopamine in severe hypothermia: Improved systolic and diastolic function, but no effect on cardiac output

The finding that DA at16 µg/kg/min at 25°C resulted in increased SVR without increasing CO, contrary to in the normothermic situation, is consistent with earlier referred reports that when epinephrine, in a dose that induced vasodilatation and elevated CO in normothermic rats, is administered during rewarming from 24°C, the result is vasoconstriction without elevation in CO (94), and that a defined dosage of epinephrine given to dogs caused vasodilatation in normothermia, but increased SVR at 25°C (95). Therefore, the altered pharmakokinetcs and pharmacodynamics of DA during severe hypothermia in the present thesis may be an effect of hypothermia on drug effects and drug metabolism common to all catecholamines.

It may seem like a paradox that DA seemed to improve isolated cardiac systolic and diastolic function in severe hypothermia, but failed to increase CO, as in normothermia or moderate hypothermia.

Would the increased SVR following high-dose DA at 25°C prevent LV systolic emptying and thus reduce SV? The answer is no, since systolic function obviously was well maintained with a DA-induced increase in dP/dt_{max} by 72% and a reduction in ESV by 48%. Also, the reduced EDV could not be explained by diastolic dysfunction, since dP/dt_{min}, Tau, and EDP were improved following DA infusion at 25°C.

The reason why DA did not elevate CO at 25°C must, at least in part, be found in extracardiac physiologic properties of the circulation, which will be discussed in the following section.

9.7 Plasma volume reduction and increased blood viscosity in hypothermia.

9.7.1 The role of cold diuresis

The phenomenon of cold diuresis is seen in awake humans subjected to cold surroundings, and starts before core body temperature falls (138;139). One factor to explain this could be nonosmotic suppression of antidiuretic hormone (ADH) in response to increasing core intravascular volume caused by peripheral vasoconstriction (78;140). Experiments on rats indicate that activation of alpha2-adrenergic receptors results in cold diuresis through inhibition of the action of ADH on renal tubuli (141). Cold diuresis does not necessarily occur in humans subjected to therapeutic hypothermia (142), possibly, among other factors, because lack of alpha2-adrenergic receptor stimulation due to deep sedation. In awake pigs however, cold diuresis was not observed (143), and it is therefore not surprising that we did not observe any cold-induced diuresis in the anesthetized pigs in Papers 1 and 2. That neither cold diuresis nor DA-induced diuresis was observed in the pigs, as opposed to in humans, is not solely a limitation of the present model; it may also be seen as an advantage: Plasma depletion in hypothermic pigs in Papers 1 and 2 has to be explained by other mechanisms that probably also play a role in humans.

9.7.2 Mechanisms of plasma volume reduction

Experimental surface cooling has demonstrated reduction in plasma volume while plasma protein concentrations have remained unaltered, which indicates a loss of whole plasma from the circulation (30;118;144). More than 50 years after the first studies on this topic were performed there is still no unifying theory to explain the mechanisms of this plasma loss. One hypothesis has stated that plasma volume probably was reduced during hypothermia because plasma was trapped in minute peripheral vessels from which erytrocytes were excluded (30). The findings of Klussmann and Lutcke in 1958 (31) and Chen and Chien in 1977 (144) also opt for the possibility that red blood cells together with plasma could be trapped in the microcirculation during surface hypothermia as they found that hypothermia-induced expulsion of erytrocytes from the spleen contributed to the increase in hematocrit during hypothermia. Representing an alternative hypothesis, Hammersborg et al more

recently have been preoccupied with extravasation of whole plasma as a cause for plasma loss in surface cooling, and that inflammation may play a part in this process (45;118). The differing views on the issue of plasma loss in surface cooling probably are not mutually exclusive, but demonstrate the complexity of fluid distribution between the intravascular and interstitial compartments during hypothermia. Whatever are the causes of this plasma loss, the process is perceived as reversible during and after rewarming (119). From a clinical point of view, intravascular hypovolemia in victims of accidental hypothermia generally is suspected during rewarming, and so administration of varm intravenous fluids is recommended as part of the rewarming process (78;79;82;100;145;146;147).

The temperature-dependent fluctuations in Hb concentration throughout the experiment in Papers 1 and 2 could not be attributed to periodic blood loss, as bleeding from instrumentation was minimal and blood sampling was evenly distributed. Also, Hb concentration changes were unrelated to diuresis, as diuretic output was constant throughout experiments. More likely, changes in Hb concentration were caused by fluctuations in plasma volume as discussed above. Our findings of temporarily decreased CVP, EDV and ESV during hypothermia support the assumption that plasma volume was reduced in hypothermia and returned to normal during rewarming.

9.7.3 Increased blood viscosity in hypothermia

Another factor that would potentially influence intravascular flow in hypothermia is the viscosity of blood. Both increased hematocrit and decreased temperature is known to increase blood viscosity (63;64). However, blood viscosity is also highly dependent of the shear rate. (63;64). Shear rate is the rate of change of velocity at which one layer of fluid passes over an adjacent layer, and it increases in proportion with fluid flow and is inversely related to the vessel diameter (148). Reduction in CO, as is seen in hypothermia, has been assumed to be proportional with reduction in shear rate (63). When shear rate is reduced, blood does not behave like a Newtonian fluid (for instance water), but greater “friction” within the blood increases blood viscosity. In vitro experiments on human blood and in vivo experiments on dogs demonstrated that at 25°C only part of the total blood viscosity could be attributed to increased

hematocrit and decreased temperature, as shear rate was found to account for a larger portion of the total increase in blood viscosity (63;64).
In sum, the most plausible explanation that DA in Paper 2 did not increase CO at 25°C despite seemingly improved DA-induced LV systolic and diastolic function, was that increased blood viscosity and reduced circulating blood volume, together with DA-induced increase in HR, precluded diastolic filling of the heart.

9.8 Is there any need for inotropic support during hypothermia?

Written guidelines for the use of inotropic medication at reduced core temperatures are scarce and based on a limited number of preclinical studies only (79). Still, studies have reported that > 50% of hypothermic patients receive inotropic drug therapy when treated with moderate therapeutic hypothermia (32–34°C) after resuscitation from cardiac arrest (101;102) or during rewarming from accidental hypothermia (18;54). In contrast, in patients hospitalized for acute heart failure without hypothermia, a subgroup of about only 10% received inotropic drugs (103).
In animals in Papers 1 and 2, CI was reduced by 70% at 25°C. However, MAP remained at 50 mm Hg, there were no signs of inadequate global oxygen supply, and diuresis was maintained. The hypothermic injury inflicted on the animals was a mild post-hypothermic systolic dysfunction that was well compensated for .
Therefore, the animals were not in need of inotropic support during or after hypothermia. It would have been a misinterpretation to give a vasoconstrictor to elevate MAP at 25°C, since the low MAP resulted from low CI and not from a low SVR, which was in fact increased as an effect of hypothermia .
The infusion of high-dose DA at 25°C in Paper 2 had no effects on CI but gave the adverse effect by increasing SVR, thus potentially compromising organ perfusion. Also, abrupt termination of DA infusion at 25°C was potentially detrimental by inducing a temporary reduction in global oxygen supply. The slowed rewarming in the DA group from 25°C to 30°C in Paper 2 could also be interpreted as an adverse effect from high-dose DA, since the combined effect of increased SVR and decreased CI after DA withdrawal may have slowed heat transfer from body surface to core.
It has been argued that inotropes in normothermic acute heart failure should

be applied on a short-term basis and confined to patients with a clear evidence of impaired organ perfusion and a low output state (149). In hypothermia, a low CO may perfectly match global oxygen requirements, since global oxygen requirements decline by about 6% per ˚C (39). To avoid excessive use of inotropic drugs in hypothermic patients, the balance between global delivery and consumption of oxygen, together with a reasonable perfusion pressure goal, should probably direct inotropic medication rather than normothermic flow end points.

In clinical medicine, accidental hypothermia patients and patients in induced hypothermia may also suffer from septic or ischemic injuries and therefore be in need of inotropic support. In such settings, drugs that elevate a subnormal SVR may be useful, as well as agents that increase SV without causing detrimental chronotropic responses and/or increased myocardial oxygen consumption. While both norepinephrine and the calcium sensitizer levosimendan are used for these purposes under normothermic conditions (149;150), they are yet inadequately tested *in vivo* during hypothermic conditions.

9.9 Limited effect of dopamine in severe post-hypothermic cardiac failure

In Paper 3, DA was administered to support a dramatically failing circulation during and after weaning from CPB at completed rewarming. While only one animal in the CPB_c group received DA after weaning, all but one of surviving animals that had been immersion cooled to HCA were given DA, at rates that averaged 7.4μg/kg/min. A DA infusion rate of 8 μg/kg/min resulted in increased HR and CO, and a decreased SVR, after rewarming in animals that were immersion cooled to 25˚C in Paper 2. None of these hemodynamic effects from DA were evident in the severe post-hypothermic heart failure in animals in Paper 3. If one should translate to the clinical situation, patients displaying such a grave post-hypothermic cardiovascular failure should probably be supported by mechanical devices like continued CPB combined with intra-aortic balloon counter pulsation rather than by inotropic drugs.

9.10 Cooling and rewarming rates and temperature afterdrop

Animals in Papers 1 and 2, and animals in the IMMc group in Paper 3, were immersion cooled at a rate of about 6˚C/hour. Factors that could explain the high cooling rate are that shivering was attenuated since animals were deeply anesthetized, the animals had a relatively large surface to weight ratio, they had no insulating clothing, subcutaneous fat layer is minimal in the applied breeds, water temperature was around 5˚C, animals were partly covered with ice slush, and heat loss was by a combination of convection and conduction since water was circulated through an external heat exchanger. In contrast, during laboratory immersion in water at 5˚C of adult humans wearing outdoor clothing, reduction of body core temperature to 35˚C takes about 1 h, which equals a cooling rate of only 2˚C/hour (151). Of course, cooling would be faster in open water due to heat loss from the head, but as a rule of the thumb it has been stated that a core body temperature drop in adults to 35˚C in outdoor immersion takes at least 30 minutes, even in cold water (152).

Immersion rewarming rate was about 6˚C/hour , which is much faster than the mean rewarming rate of 1.7˚C/hour reported by rewarming of hypothermic victims by forced air (54).

During immersion cooling to 25˚C (Papers 1 and 2), a further temperature drop was prevented by emptying cold water from the tub at 26˚C during cooling, and by adding small amounts of hot water to the tub. In this way, the afterdrop from 26 to 25˚C was made an integral part of the cooling. In a clinical setting, observing a temperature drop in a victim after removal from cold water into warm surroundings would have been referred to as regular afterdrop. Experiments on volunteers that were removed from cold water (8˚C) have demonstrated that temperature afterdrop lasted for about 30 minutes and amounted to be about 1˚C (86). Given good prehospital care, afterdrop should ideally have ceased by admission to hospital, and no further temperature fall should be observed after start of active rewarming, as demonstrated in the clinical study by Kornberger et al (54). In Papers 1 and 2 afterdrop was not observed during the rapid surface rewarming.

The fact that afterdrop occurs even after circulatory arrest was demonstrated in Paper 3, where immersion cooled animals continued to cool in all measured body

compartments during 75 min of HCA (Fig. 1A). In contrast, animals that were core cooled, that is cooled by CPB, had what may be called a temperature “after-rise”: Temperature curves from all measured body compartments inclined upwards during 75 min of HCA (Fig. 1B).

All temperature drifting observed in animals in Papers 1-3 may be understood within the framework of simple thermo-dynamics, that is, transfer of heat between core and surface along temperature gradients, as outlined in section 5.5.5.

10. FINAL CONCLUSIONS

10.1 Paper 1

- Cardiac left ventricular contractility decreases during surface cooling below 34°C.
- Cardiac systolic function is depressed after rewarming from 25°C.
- Cardiac diastolic function is restored after rewarming from 25°C.
- Post-hypothermic cardiac output is maintained by a spontaneous increase in heart rate.
- Decreased post-hypothermic systemic vascular resistance may be adaptive measure.

10.2 Paper 2

- Pharmacokinetics of DA normalized during rewarming at moderate hypothermia (30 - 34˚C). Effects of DA during moderate hypothermia were comparable to the normothermic situation.
- Pharmacokinetics of DA was seriously altered at severe hypothermia (25˚C). Even if DA improved both systolic and diastolic function, cardiac output did not improve since stroke volume decreased with incrementing DA dosages.
- Increased SVR at high-dose DA at 25°C suggests vascular α-adrenergic involvement not seen in normothermia.
- Properties of the low-flow, high-viscosity circulatory state, combined with serious alterations in the pharmacokinetics of DA, may explain the lack of beneficial – and potentially harmful – effects from DA administration at 25°C.
- Administration of DA during hypothermia did not blunt post-hypothermic responses to DA.
- Administration of DA during rewarming did not increase rate of rewarming.
- Temperature-related alterations in pharmakokinetcs and effects of DA in the present thesis confirm similar results from testing epinephrine in other species and may be a common effect of hypothermia on catecholamines.

- Our findings support guidelines from American Heart Association (2010) and European Resuscitation Council (2010) to maintain an attitude of reservation towards administration of catecholamines if core body temperature is < 30°C

10.3 Paper 3

- Immersion cooling below 25ºC resulted in deteriorated global perfusion and oxygen delivery.
- Regional ischemic damage may have taken place in the brain and other vital organs in the IMM_c animals during immersion cooling below 25ºC .
- Immersion cooling to deep hypothermic circulatory arrest followed by 75 min resulted in severe cardiovascular failure after rewarming.
- Autoregulatory reflexes to compensate for cardiovascular failure were absent in animals surviving rewarming after immersion cooling to HCA.

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Paper I

Paper II

Paper III

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