HYPOTHERMIA AND TRAUMA

by

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Panta rei!

Herakleitos of Ephesus (540-480 B.C.)

To **Isabella & Simon**

LIST OF PUBLICATIONS

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

I.   Wladis A, Hjelmqvist H, Brismar B, Kjellström BT. Acute metabolic and endocrine effects of induced hypothermia in hemorrhagic shock - an experimental study in the pig. *J Trauma* **45**: 527-533, 1998.

II.   Wladis A, Hahn R, Hjelmqvist H, Brismar B, Kjellström BT. Acute hemodynamic effects of induced hypothermia in hemorrhagic shock - an experimental study in the pig. *Shock* **15**:60-64, 2001.

III.   Wladis A, Hahn R, Brismar B, Kjellström BT. Effects of induced hypothermia after soft-tissue injury. (Submitted)

IV.   Wladis A, Hahn R, Brismar B, Kjellström BT. Induced hypothermia after high-energy soft-tissue injury and subsequent hemorrhagic shock. (In press)

V.   Heinius G, Wladis A, Hahn R, Kjellström BT. Induced hypothermia and rewarming after hemorrhagic shock. (Submitted)

ABSTRACT

Hypothermia and trauma

Background Accidental hypothermia (HT) has been found to increase morbidity and mortality in trauma patients. In contrast, HT has been induced in certain surgical procedures for several decades because of its cerebroprotective properties. HT has even been used therapeutically in patients with traumatic brain injury. In recent years, a number of experimental studies have suggested beneficial effects of induced HT in hemorrhagic shock (HS), but just how induced HT affects the organism subjected to both HS and trauma has been unknown, hitherto.

Methods In papers I and II, animals were exposed to 50% exsanguination during 25 min. In paper III, a standardized gunshot wound was inflicted on the right hind-leg. In paper IV, animals were subjected to the combination of these insults. In paper V, the hemorrhage amounted to 40% of the blood volume and was achieved in 3-5 min. Core temperature, electrolytes, arterial blood gases, blood cell counts, Hb, and central hemodynamics were monitored in all the studies. Catecholamines were analyzed in papers I-IV. IL-6 was studied in papers III-IV. Thromboelastography was used to evaluate coagulation abnormalities in paper V. In this paper, animals were rewarmed after cooling.

Results Paper I: Catecholamine levels in plasma increased in response to the hemorrhage, but gradually decreased with cooling. Serum potassium levels increased in the controls, but decreased transiently in HT animals. Paper II: HR increased markedly after the hemorrhage, while CO and MAP were reduced. With HT, HR decreased and CO and MAP were further depressed. Leukocyte counts decreased in HT animals. Paper III: HR, MAP, neutrophil granulocyte counts and plasma adrenaline levels were lower in the HT group. Cardiac index decreased slightly in both groups. Serum potassium increased with normothermia, but was not affected in HT pigs. Paper IV: HR, VO_2, ER, serum potassium, and creatinine levels were lower with cooling. Paper V: VO_2 was reduced in HT animals. Serum levels of potassium were transiently stabilized with HT. The formation of blood clots was delayed, but once formed, the clot strength was unaffected by HT. Effects of HT were reversed with rewarming.

Conclusions In HS and/or soft-tissue trauma, HT reduced plasma catecholamine levels and transiently stabilized serum levels of potassium. Central hemodynamics after the combination of the insults was affected by HT to a remarkably small extent, while VO_2 and ER decreased. In the presence of rewarming hemodynamics, VO_2 and ER regained baseline levels.

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CONTENTS

ABBREVIATIONS

BW	Body weight
CaO_2	Arterial oxygen content
CO	Cardiac output
CI	Cardiac index
CvO_2	Mixed venous oxygen content
CVP	Central venous pressure
DO_2	Oxygen delivery
ER	Oxygen extraction ratio
GSW	Gunshot wound
Hb	Hemoglobin concentration in blood
HR	Heart rate
HS	Hemorrhagic shock
HT	Hypothermia, hypothermic
IL-6	Interleukin-6
i.v.	Intravenous(ly)
i.m.	Intramuscular(ly)
MAP	Mean arterial pressure
MPAP	Mean pulmonary artery pressure
NT	Normothermia, normothermic
PCWP	Pulmonary capillary wedge pressure
PO_2	Arterial oxygen tension
SaO_2	Arterial oxygen saturation
SEM	Standard error of the mean
SV	Stroke volume
SvO_2	Mixed venous oxygen tension
VO_2	Oxygen uptake

DEFINITIONS

Accidental HT	Inadvertent HT
Induced HT	Willfully induced HT
Therapeutic HT	HT induced for therapeutic purposes
Oxygen delivery (DO_2)	$CO \times CaO_2 / BW$
Oxygen uptake (VO_2)	$CO \times (CaO_2 - CvO_2) / BW$
ER	VO_2/DO_2
CaO_2	$(SaO_2 \times Hb \times 1.39) + (0.223 \times PaO_2)$
CvO_2	$SvO_2 \times Hb \times 1.39$
Systemic vascular resistance (SVR)	$(MAP-CVP) \times 79.98/CO$
Pulmonary vascular resistance (PVR)	$(MPAP-PCWP) \times 79.98/CO$

INTRODUCTION

Trauma is, by definition, an injury of any sort, but it is usually referred to as a somatic injury severe enough to pose a threat to life or limb[1]. Such injuries often require immediate attention or else they worsen or even cause death. In Sweden and other countries in the West, trauma represents a major cause of preventable death in persons under the age of 44 and causes a greater loss of life years than do cardiovascular ailments and cancer taken together[2].

A reduction of the body core temperature, HT, either preserves life or kills[3]. The exact mechanism behind these two main effects remains unknown. Before any of these effects are reached, HT will have altered a broad range of physiological parameters in different directions depending on how and when HT is achieved and for how long it is maintained.

This thesis is focused on the effects of induced HT in three forms of trauma: HS, soft-tissue injury, and the combination of these. It also deals with how the HT-induced effects on HS are altered during rewarming.

Historical notes

Accidental hypothermia

One of the oldest accounts of HT can be found in the Old Testament of the Bible (1 Kings, 1:1-2):

> When King David was old and well advanced in years, he could not keep
> warm even when they put covers over him. So his servants said to him, "Let
> us look for a young virgin to attend the king and take care of him. She can
> lie beside him so that our lord the king may keep warm."

Throughout history, accidental HT has frequently been reported in conjunction with military battles, and cold weather has altered the course of many of them. Accordingly, Hannibal is said to have lost about 20,000 soldiers in 218 B.C. while en route through the Alps. A month after the Swedish King Carolus XII had fallen in the battle of Frederikshald, Norway, in 1718, about 5000 of his men attempted to return to Sweden. They got caught in blizzard and around 3000 soldiers succumbed due to HT and/or frostbite.

Moreover, in 1812, the French Emperor Napoleon I lost most of what remained of his Grande Armée due to the cold while attempting to invade Moscow. Baron Dominique Jean

Larrey, Emperor Napoleon's surgeon-in-chief made the observation in his memoirs that soldiers sitting closest to the fire died "mysteriously", which may be the first account of shock induced by external rewarming of HT subjects [4].

More recently, the French lost more than 1000 soldiers in 1845-55 in the Crimean War due to HT. The battle of Stalingrad in 1942-43 was not only one of the greatest and most atrocious struggles in the history of warfare, but also a turning point in World War II. It cost more lives than any other battle: 1.1 Million Russians and 800,000 Germans. Many of the German casualties were due to HT and frostbite.

Therapeutic hypothermia

The use of cold for therapeutic purposes is described in the most ancient medical text known hitherto, the Edwin Smith Papyrus (ca. 3500 B.C.)[5]. Furthermore, the Chinese surgeon Hua T'O (ca. 200 A.D.) used general HT for chronic fever[5]. Baron Larrey, Emperor Napoleon's surgeon-in-chief, is known to have advocated the use of snow massage as a means of treating local HT (frostbite). This treatment, as paradoxical as it may sound, was widely adopted and was in use in the Royal Swedish Army well in to the 20th century. In 1932, a textbook from the medical services of the Royal Swedish Army recommended not only snow massage but, indeed, a bath in ice-cold water to treat general HT[6].

Induced hypothermia

Induced HT was introduced in clinical practice close to 50 years ago to provide whole-body protection, and cerebral protection in particular, while enabling successful surgical correction of cardiac anomalies in children. Initially, deep HT was achieved by means of surface cooling or core cooling, or a combination of these. The aim was to decrease the core temperature and cerebral metabolism enough to allow periods of circulatory arrest without incurring cerebral injury. These periods were kept as short as possible to minimize morbidity and mortality. Presently, the use of HT circulatory arrest has expanded to include surgical interventions for cerebral and complex aortic aneurysms in adults, reoperations for cardiac valve replacements, hepatic tumor resection, and operations for tumors within the venae cavae[7].

Hemorrhage, shock and trauma

Shock arises when the perfusion of vital organs is not sufficient to satisfy the metabolic demands of cells. Shock is the clinical manifestation of a trauma too severe to be immediately

compensated for by the body's regulatory mechanisms and may result in death without expeditious treatment. Seven categories of shock have been proposed: hypovolemic, vasogenic, neurogenic, septic, cardiogenic, obstructive, and traumatic[8]. Weil and Shubin, in turn, defined four categories of circulatory shock, namely: hypovolemic, cardiogenic, obstructive, and distributive[9].

Except for septic shock, shock is characterized by a decrease in systemic oxygen transport due to hypovolemia and myocardial or respiratory failure. In septic shock, there is a maldistribution of nutritive organ blood flow owing to microcirculatory disturbances in spite of normal or even increased CO. Common to all forms of shock is a severe derangement of local perfusion because of activated leukocytes and the release of humoral mediators which cause changes in organs not primarily affected by the initiating insult[10]. Two of the forms of shock, hypovolemic and traumatic shock, are relevant in this context and will be discussed below although it is recognized that hypovolemic shock in real life is oftentimes compounded by *e.g.* neurogenic, cardiogenic, or obstructive shock.

Hypovolemic and hemorrhagic shock

In most cases, hypovolemic shock is caused by hemorrhage and the expression is therefore often interchangeable with HS. However, hypovolemia may also arise from other conditions, such as severe gastrointestinal, renal, or cutaneous fluid loss. It can also be caused by excessive ascites formation. Moreover, a situation with relative hypovolemia may result from vasodilation. For the sake of clarity, only the expression HS will therefore be used in the following.

It is noteworthy that HS may arise without overt bleeding, such as when blood accumulates in the intestine, muscles, or retroperitoneum. Thus, HS may be due to either external or internal bleeding. Some common examples of externally induced HS are traumatic amputation of a limb, disruption of blood vessels, excessive blood loss during surgery, while internal causes of HS include extrauterine pregnancies, esophageal and gastrointestinal hemorrhage, traumatization of parenchymatous organs, and fractures of major bones.

Exsanguinating hemorrhage is the cause of death in 31% of civilian and 47% of military trauma victims [11]. Half of all trauma deaths occur within minutes of the injury. They are usually due to severe injuries to the brain or major vessels and are only rarely susceptible to curative treatment. Approximately 30% of trauma-related mortality occurs within the first few hours and is caused by neurological injuries or various kinds of hemorrhage [2]. The concept of

a "golden hour" following injury arises from considerations of this group. But in spite of standard resuscitative measures, the mortality rate remains high.

Pathophysiology of hemorrhagic shock

The balance between oxygen delivery and oxygen demand is maintained as long as tissue oxygen extraction can increase in cases of reduced blood flow. At a certain point, tissue perfusion becomes inadequate for the cellular oxygen needs. Oxygen debt thus materializes, resulting in anaerobic metabolism, cellular acidosis, and lactic acidosis.

Acute hemorrhage causes redistribution of the blood flow to preserve perfusion and the viability of vital organs. Accordingly, the blood flow to the skin, musculature, and the splanchnic region is considerably reduced. The high adrenergic innervation of the splanchnic vascular region will cause the blood flow to the intestine and its mucosa, in particular, to be severely compromised when the sympathetic nervous system is activated in response to the hemorrhage. In a study on monkeys in HS, the intestines still remained underperfused 2 h after the blood volume had been restored [12]. This intestinal ischemia may cause endotoxemia and bacterial translocation and trigger massive production and release of hepatic cytokines, which, in turn, might eventually contribute to the development of multiple organ failure. However, the data on bacterial translocation after HS are inconsistent and its clinical significance remains unclear[8].

The physiological hemodynamic response to major hemorrhage is aimed at preserving the blood circulation to vital organs, restoring blood loss, and limiting the hemorrhage. In principle, this response often evolves as follows:

1) Decreasing circulating blood volume is detected within seconds by arterial baroreceptors which sense decreased stretching of the arterial wall. This releases the chronic inhibition imposed by the baroreceptors, which causes the nucleus tractus solitarius in the medulla oblongata to reduce the tonic inhibition of the HR. Also, by virtue of activation of the sympathetic outflow with noradrenaline release from postganglionic sympathetic fibers, constriction of venous capacitance vessels is achieved. This, in turn, increases the venous return to the heart and thus maintains the CO and MAP[13, 14]. In addition to the sympatho-adrenal axis, both vasopressin and angiotensin II may contribute within 10 min to an hour to maintaining perfusion during hemorrhage[14, 15]. The magnitude

of the neuroendocrine response to hemorrhage depends on the magnitude of the decrease in effective circulating volume and on the rate at which the blood loss occurs. The neuroendocrine reaction may also be modified by associated injuries or conditions, drugs, prescribed medication, pre-existing illness, drug withdrawal, the age of the patient, pain, and psychological factors such as fear and stress. Moreover, repeated insults may also potentiate the response[8].

2) The posthemorrhage activation of the sympathetic nervous system also causes a noradrenaline-mediated constriction of precapillary arterioles, which decreases the capillary hydrostatic pressure. Up to 0.5L of fluid from the interstitial space can be mobilized in the adult and drawn into the capillaries to compensate for parts of the blood loss in this fashion. Adrenaline released into the circulation stimulates hepatic glycogenolysis, leading to hyperosmotic hyperglycemia, which may mobilize another 0.5L of cellular water into the bloodstream[16]. Eventually, the compensatory mechanisms are exhausted and the venous return to the heart decreases. At this point, maintenance of normal CO is no longer possible and the MAP decreases. With decreasing MAP, the coronary blood flow decreases and thus a vicious circle is entered.

3) The ischemic hypoxia that comes with hemorrhage and the release of circulating cytokines and other mediators cause swelling of the endothelial cells, which impedes perfusion by narrowing the vessel lumen. This sludging phenomenon is aggravated by the adherence of activated leukocytes, mainly neutrophils, to the vascular endothelium[10].

HS is a dynamic process in which the relevant variables change depending on the degree of hemorrhage and achieved resuscitation. In order to simplify and stratify the characterization and treatment of HS, some authors have divided it into a number of stages. AC Guyton, for one, described three stages, the first one named nonprogressive or compensated, the second progressive or decompensated, and the third irreversible [14]. The first one is called so because the neuroendocrine responses are sufficient to compensate for the hypovolemia. If this compensation is insufficient, organ and cellular dysfunction will follow

and decompensated chock will occur. Organ and cellular dysfunction can still be reversed by appropriate volume resuscitation. It is not known exactly what causes the transition from the compensated to the decompensated stage, but local factors such as acidosis and adrenergic activity are known to be able to alter the microvascular response. Consequently, vasodilation occurs with a decrease in peripheral vascular resistance and a decrease of venous return to the heart with a further decline in CO and increasing tissue acidosis. If volume loss continues or fluid resuscitation is inadequate, irreversible shock will occur insidiously. This is usually defined in retrospect[8].

Peter Baskett has chosen another way of classifying HS based on the estimated volume of blood lost with four, partly overlapping, stages as a guideline for the treatment of patients in HS[17]. According to this system, patients with a blood loss up to 15% of the calculated blood volume (*i.e.* up to 750 ml in a 70kg patient) belong to class I. The corresponding figures for class II is 15-30%, for class III, 30-40%, and, for class IV, >40%.

Trauma and traumatic shock

Traumatic shock should be considered a separate entity, as it comprises components of several of the aforementioned types of shock. In its purest form, however, it involves HS in combination with soft-tissue trauma and/or bone fractures. Consequently, the study of pure HS has limited relevance to the pathophysiological condition of traumatic shock. Pulmonary complications, *e.g.* after HS, are uncommon in clinical practice, while in traumatic shock they are common, probably due to post-injury release of various proinflammatory mediators[8]. Such a release is more intense in traumatic shock than in pure HS.

The treatment of hemorrhagic shock

The treatment of HS is directed toward restoring organ perfusion with adequately oxygenated blood. This is achieved by rapidly securing an airway, replacement of the blood volume, and by prompt control of the hemorrhage. The current dogma dictates resuscitation with asanguinous fluids in addition to packed red blood cells[18]. The choice of asanguinous fluid, be it crystalloid or colloid, is a matter of scientific discussion and lies beyond the scope of this thesis[16]. This is also true of hypertonic saline solutions.

In uncontrolled hemorrhage, some authors advocate that i.v. fluid be withheld until the patient has arrived in the operating room. This stance is based on data showing increased survival with fluid restriction in the field after penetrating thoracic injuries[19]. It is believed that the differences between the groups develops because of rebleeding in patients receiving

i.v. fluid before surgery. These notions are not yet generally accepted and apply, at any rate, only to patients with penetrating traumas, ruptured abdominal aortic aneurysms, or gastrointestinal bleeding. Patients subjected to blunt trauma and possibly head injury should still receive standard resuscitative treatment[8].

Thermophysiology and hypothermia

Thermophysiology

Man is homeothermic (warm-blooded) as are most mammals, which means they strive to maintain a constant body core temperature within a narrow range regardless of changes in the ambient temperature. A few animals hibernate, which causes their basal metabolic rate to be considerably reduced for a period of time.

Maintenance of the core temperature is governed by the hypothalamus, which receives input concerning the thermal condition of the body. This information, which originates from thermosensors in the skin, various core regions, and locally in the hypothalamus, is compared with a set point signal, after which appropriate output commands are activated. A number of factors affect the regulated set point: time of day, sleep state, activity levels, presence of pyrogens, ambient temperature, and satiety. Information about the ambient temperature is extremely important, if not the most important, for temperature homeostasis[20]. Consequently, the skin temperature has a big impact on the behavioral thermoregulatory responses, but a smaller effect on autonomic thermoregulation. However, a low skin temperature in conjunction with immersion in cold water can also elicit a strong and more or less immediate response from the sympathetic nervous system[21].

The preoptic anterior hypothalamic area of the hypothalamus (POAH) has been identified as the center for autonomic thermoregulation[20]. A decrease in the temperature of blood flowing in the POAH will elicit vasoconstriction and increase metabolic heat production. Conversely, adding heat to the POAH will stimulate vasodilation and sweating and thus decrease the body core temperature. The magnitude of the thermoregulatory response is proportional to the displacement of the POAH temperature from the threshold temperature.

The blood circulation in the skin is maintained by two types of vasculature. These are (1) nutritive vessels consisting of arteries, veins, and capillaries and (2) heat exchange vessels consisting of venous plexuses and arteriovenous anastomoses. While the nutritive vasculature is found uniformly in the skin, the heat exchange vessels are found only in the hairless skin of the hands, feet, ears, and face. It has been estimated that the blood flow in to the venous

plexuses can range from near zero to as much as 30% of the total CO. With maximal vasoconstriction of these vessels, the body core is insulated from the environment[22].

Normally, behavioral adjustment and autonomous subcutaneous heat exchange are sufficient to maintain thermal homeostasis in man. Heat for thermoregulation is lost by sweating and produced by two mechanisms, shivering and non-shivering thermogenesis[20, 23]. The latter is only seen in infant humans, making shivering thermogenesis the main heat producer. Shivering is the action of muscular agonists and antagonists acting in a non-synchronized manner. It leads to tremor but little other movement of the body. In a resting human, about 20% of the heat is generated by the muscles. With maximum shivering, however, the muscular heat production can increase manifold, causing the metabolic rate to increase two to five times and will increase O_2 consumption by 40-100%[23-25].

The human or porcine body is thermally compartmentalized. The thermal core consists of the organs that generate heat, whereas the thermal periphery are tissues engaged in heat exchange and tissues underlying the body surface. Such tissues can include subcutaneous fat and skeletal muscle. One of the functions of these tissues is to buffer the thermal core from environmental attempts to lower the body core temperature[22]. Differences in this insulation can determine the occurrence of HT and the rate of heat loss. At low ambient temperatures, the thermal core, which is not an anatomical entity, can shrink and eventually only encompass critical organs. The central nervous system, being the center of thermoregulation, is the most protected of the core organs and thus the last region of the body to lose NT. In contrast, the normal temperature of the skin is approximately 22-23°C.

Hypothermia

HT is commonly, but arbitrarily, defined as a general decrease of the body core temperature below 35°C, regardless of whether the temperature decrease is accidental or induced[26, 27]. The severity of human HT is classified in different ways depending on the patient's core temperature. The most common classification defines mild HT as being between 32 and 35°C, while moderate HT is between 28 and 32°C and severe HT is below 28°C[27]. This classification mainly follows some physiologically important steps. Accordingly, shivering is most intense at 35°C and it ceases at about 32°C. Cardiac arrhythmias usually appear around a core temperature of 30 to 28°C.

The physical laws governing heat loss are critical to the understanding of the development, management, and prevention of HT. Hence, heat loss occurs in four ways:

conduction, the transfer of heat by direct contact, particularly important in cold water immersion where thermal conductivity is 32 times that of air; *convection,* the transfer of heat by particles of air or water that have been heated by contact with the body; *radiation*, the transfer of heat by non-particulate means, such as heat from unprotected skin; and *evaporation* of water.

As mentioned previously, HT either kills or preserves life[3]. The latter effect is created by the HT-induced decrease in metabolic activity, and hence in VO_2. The survival time of HT mammals is rather long in mild HT, but it is greatly shortened when the core temperature is profoundly reduced.

The abnormal physiology of HT depends on many factors, such as how it is attained, at what speed this occurs, and the depth and duration of HT. The general condition of the organism will also have influence on the effects of HT. Infants and the elderly, *e.g.*, are more sensitive to HT and do not have the same capability to cope with a decrease in the core temperature. Likewise, several drugs and medical conditions, such as diabetes, hypothyreosis, burn injuries, injuries to the central nervous system, HS, and trauma, can adversely effect the thermoregulatory response. Moreover, it is a well-known fact that there are species differences in the reaction to HT. Small animals are more sensitive to low ambient temperatures and become HT easier, but, paradoxically, appear to be more tolerant to HT[28].

Some of the major physiological reactions to HT in man are listed below:

Metabolic rate

If shivering is prevented, tissue VO_2 and the metabolic rate will decrease progressively with decreasing core temperatures. Hence, at a core temperature of 32°C, the metabolic rate will have fallen by 25%. At 28°C the corresponding figure is 50%[23]. In cases of uninhibited shivering, however, the metabolic rate has been reported to increase by up to 600%, but more recent studies suggest that this increase is normally around 100% in shivering patients postoperatively[29, 30].

Central nervous system

Drowsiness and dysarthria will occur at around 33°C. At 30°C, the patient is usually stuporous, but can be readily aroused[23]. At 26°C and below, the patient will often fail to

respond to any stimulus. At this level, patients will often also lose their normal reflexes. Electroencephalographic activity will disappear completely at 20-15°C[24].

Hemodynamics

The HR and MAP will often increase somewhat initially in response to cold[24]. This is due to the immediate sympatho-adrenal reaction to cold exposure and may also result from shivering. HT reduces the myocardial work, but the myocardial efficiency is increased so that the bradycardia is still sufficient for myocardial function. The myocardial work is, however, increased by the vigorous systemic vasoconstriction that accompanies HT. Consequently, CO will be reduced because of bradycardia and increased peripheral resistance. There is a prolongation of the isovolumetric relaxation phase and the conduction velocity. The decrease in CO during HT is proportional to the decrease in myocardial oxygen consumption[31].

Heart rhythmicity

With progressing HT, higher centers of rhythmicity are suppressed, causing lower pacemakers to act[23]. Hence, sinus rhythm may be followed by atrial flutter or fibrillation. Ventricular fibrillation may appear at core temperatures below 30°C. This often fatal arrhythmia may be precipitated by physical handling of the patient, such as endotracheal intubation[27]. The cause of ventricular fibrillation in HT remains unknown. Around a core temperature of 20°C, asystole will occur.

At around 31°C, the QRS complex will begin to widen at the base and the QT interval will be prolonged. Eventually, an inversion of the T-wave may develop, often accompanied by the characteristic J-wave or J-deflection, which is a secondary wave at the QRS-ST junction[24]. These ECG findings are, however, often inconsistent.

Renal function and fluid balance

HT has a depressant effect on all aspects of renal function and, at 30°C, the renal blood flow has decreased by as much as 50%, but the HT-induced reduction of tubular reabsorption of sodium and water may be the dominant effect and it is due to the reduced oxidative activity[23]. This may cause polyuria, especially in conjunction with so-called cold diuresis, which is due to an increased blood volume centrally after peripheral vasoconstriction[27]. Consequently, hypovolemia will develop. In addition to this, increased levels of

catecholamines may cause a translocation of fluid to the interstitial space, further exacerbating the hypovolemia[24].

Blood chemistry

Electrolyte changes appear to vary greatly depending on the circumstances around the HT. Either hyperkalemia or hypokalemia has been described, whereas hyponatremia is seen most frequently[24].

Platelets and leukocytes are sequestered in the spleen or the liver, causing thrombocytopenia and leukopenia[27]. HT-induced bone marrow depression may enhance this effect.

The adrenergic response to cold stress appears to be inconsistent. Some authors report that cold exposure in humans causes sympatho-adrenal response with increasing levels of plasma catecholamines[21, 32, 33]. Lehot and coworkers also reported an increase in plasma catecholamines in patients on cardiopulmonary bypass, but the increase in the NT group was twice as high[34].

In contrast, plasma catecholamines decreased after the induction of HT in some animal studies[35-37].

Respiratory function and acid-base status

The respiratory rate may increase transiently as an immediate reaction to cold exposure, but it soon falls progressively with the core temperature. Below 28°C, the respiratory rate may be less than 4 breaths/min[24, 27].

The oxyhemoglobin dissociation curve shifts to the left with decreasing temperature and PO_2 and CO_2 decrease about 5% per °C decrease in core temperature, which is in line with the decrease in the metabolic rate in HT[27]. In accidental HT, however, when shivering is uninhibited, acidosis may develop because of depression of the respiratory drive in conjunction with deteriorated peripheral circulation, tissue hypoxia, lactate formation and ketogenesis in the liver.

Coagulation and blood viscosity: The function of coagulation factors and platelets decreases progressively with HT below 34-35°C. As mentioned above, thrombocytopenia often occurs in HT and contributes to the coagulopathy as well. Also, blood viscosity

increases gradually with decreasing core temperature, but it is also dependent on the blood flow[27].

Studies concerning these effects are often difficult to analyze as some of them were performed *in vitro* and in many of them the blood tests were analyzed at 37°C. One study by Schmied and coworkers, found the intra- and postoperative blood loss to be greater in inadvertently HT patients undergoing total hip arthroplasty[38]. In contrast, Johansson et al. failed to demonstrate increased blood loss in mildly HT patients subjected to the very same procedure[39]. Furthermore, Marion and coworkers did not find increased bleeding or clinically important coagulopathies in patients with traumatic brain injury treated with 33°C HT[40]. Moreover, another study on trauma patients evaluated with thromboelastography found *hypercoagulability* in patients with mild HT down to 34°C, while coagulopathy was described in core temperatures below 34°C[41].

Hypothermia in clinical practice and research

In civilian life, accidental HT usually occurs involuntarily in conjunction with accidents and in the hospital setting during surgery. Accidental HT is usually regarded as harmful and measures are normally taken to prevent or counteract it. In trauma patients, the severity of HT has been found to correlate with negative outcomes[42]. Between 21 and 50% of severely injured trauma patients become HT[43]. This is due to exposure in the field or the hospital with inadequate clothing, hypovolemia that impairs thermoregulation, common standard treatment with infusion of cold fluids and opening of body cavities, and limited heat production due to anesthetic agents, which may decrease heat production by as much as 20%[43, 44].

As mentioned previously, induced HT was introduced in clinical practice close to 50 years ago to facilitate and enable certain cardiothoracic surgical procedures in children because of its cerebroprotective properties[45]. Since then, the use of induced HT has become a normal and unchallenged part of many cardiothoracic procedures. In the most common cardiothoracic procedures, such as coronary artery bypass grafting, HT at around 32°C is used, but HT down to 18°C is used routinely in conjunction with circulatory arrest in the resection of aortic arch aneurysms[46]. It has also been used in certain interventions in pediatric surgery and neurosurgery[47, 48].

The therapeutic use of HT has been studied since the first decades of the 20th century. Most experiments were performed on animals, but Nazi doctors also studied the effects of HT on prisoners incarcerated in, for instance, the Dachau concentration camp. In the 1940's, some

clinicians also tried HT unsuccessfully as a therapeutic tool in the treatment of cancer and psychosis[28]. Nowadays, therapeutic HT is mainly used topically as a local anesthetic in minor surgical procedures, such as the removal of birthmarks.

During the past two decades, an increasing amount of experimental data has suggested that HT may be beneficial in HS and cerebral ischemia. In humans, HT has been shown to improve survival in sepsis-related adult respiratory distress syndrome[49] and in a randomized, controlled study on 82 patients with traumatic brain injury, induction of moderate HT (33°C) seemed to hasten neurological recovery and improve the outcome[40]. However, this conclusion was questioned in a multicenter study of 392 patients exposed to closed head injuries and treated with HT in a similar fashion[50]. One possible explanation of the results in the latter study may be the fact that HT patients were found to be significantly more intoxicated with alcohol, which is known to cause vasodilation with secondary heat loss as a result, and they were given significantly more i.v. fluid.

The effects of HT on homeothermic mammals thus appear to be complex and inconsistent. When evaluating these effects, one must consider the general health of the subject exposed to HT. The modality of HT and its depth and duration have considerable influence on the metabolic response. In experimental conditions, anesthetic compounds may also inhibit the thermoregulatory mechanisms.

The treatment of accidental hypothermia

When HT occurs inadvertently, it can be treated by external and/or internal warming. The method of choice depends not only on the degree of HT but also on the available facilities. In conscious patients with core temperatures above 31°C, external, passive rewarming with insulating blankets, a high ambient temperature, and warm fluids is usually sufficient. In patients with core temperatures below 31°C, active warming should be instigated. This can be done externally with warm blankets and by bathtub heating. It can also be achieved internally in many ways: warm air inhalation, warmed i.v. fluids, irrigation of the gastrointestinal tract, pleural or peritoneal lavage, and cardiopulmonary bypass[24].

The rationale for inducing hypothermia in shock and trauma

In HS, there is an inherent imbalance between the supply and demand of oxygen. As described above, this will induce anaerobic metabolism, acidosis and, ultimately, death. The standard resuscitative approach has been to uphold tissue perfusion and control the

hemorrhage. This is often, but far from always, successful. Theoretically, a reduction of the metabolic rate would alleviate the metabolic imbalance by decreasing the demand for oxygen. The disturbance of the microvascular perfusion, caused by leukocyte interaction with the endothelium, is one of the main reasons for this imbalance[10]. Thus, if the oxygen demand *and* the leukocyte count were decreased, it is conceivable that the imbalance could be shifted in a favorable direction.

AIMS OF THE STUDIES

The overall aim of this thesis was to investigate the effects of induced moderate HT superimposed on pigs in HS with and without attendant soft-tissue injury. In reality, HT often occurs after serious trauma with subsequent hemorrhage, but there are no published studies to answer the important questions raised by this clinical dilemma. Does moderate HT under these circumstances ultimately worsen the chances for survival?

Specifically, the following questions were addressed in the studies:

I.   What effect does induced moderate HT have on the acute metabolic and endocrine response to HS? Will HT exacerbate or improve the metabolic reaction to the shock? In what way will HT influence the catecholamine response to the shock?

II.   What effect does induced moderate HT have on the acute central hemodynamic response to HS? Will induced HT improve cardiovascular variables? Will induced HT even improve survival rates?

III.   What are the acute effects of induced moderate HT on hemodynamic, metabolic and endocrine parameters of soft-tissue trauma?

IV.   What are the acute hemodynamic, metabolic, and endocrine effects of moderate HT when induced after soft-tissue trauma and immediately subsequent HS? Will the attendant trauma diminish the effects of induced HT? Will HT affect survival?

V.   What effects does induced mild HT have on the general response to HS and how is this response altered by rewarming? Will HT-induced changes in studied variables return to baseline with rewarming? How effective is rewarming with an HT bed and bladder irrigation? Will HT negatively affect the formation and strength of blood clots? Will HT and/or rewarming affect survival?

METHODOLOGICAL CONSIDERATIONS

The experimental procedures and relevant technical details are presented and referred to in the separate papers. Therefore, only specific aspects concerning the animals, the surgical preparation, the hemorrhage, the induction of HT and rewarming, the trauma model, and the hemodynamic measurements will be commented on.

Animals

Porcine models have been used extensively in the past decades to study hemodynamics in general and HS in particular [51]. Earlier canine models have gradually been replaced with porcine equivalents in many laboratories since porcine hemorrhage models appear to be superior to canine models in terms of human applicability. Most circulatory functions in the pig are fully developed at birth, making the use of 2 to 3-month-old piglets suitable as models for hemorrhage and shock as well as for newly developed resuscitation procedures[51].

All pigs were of Swedish landrace weighing 17-27 (22,1) kg and were obtained from a commercial breeder. The experimental protocols were all approved by the Ethics Committee on Animal Research in Umeå, Sweden.

Fifteen of the 24 animals in papers I and II were used jointly.

Anesthesia & ventilation

Ketamine, which is known to increase HR and cardiac work in man and experimental animals alike, was chosen as the main anesthetic during the experiments to avoid the vasodilatory effects of general anesthesia[52]. Another reason for choosing ketamine was the determination to minimize polypharmacy while securing analgesia and anesthesia in the animal.

All animals were premedicated with i.m. injections (40mg/kg BW) of ketamine hydrochloride (50mg/ml) while in the pen. This dosis is relatively high, but it was deliberately chosen to diminish the need for other sedatives[53]. After 5-15 min of tranquility, the pigs were immobilized and sedated enough to be brought into the operating room and an i.v. cannula was inserted into a superficial auricular vein. This allowed i.v. administration of atropine and pentobarbital sodium to facilitate endotracheal or tracheal intubation. The typical dose of pentobarbital was 10 mg/kg BW, which is less than half of what some authors reported[54]. In

addition to this, 2.5 mg diazepam was given i.m. to animals described in papers I, II, and III. This dose of diazepam is rather low compared to the suggested dose of 2mg/kg i.m.[53].

After preparation, the maintenance dose of ketamine averaged approximately 10-12 ml/h, which corresponds to 500-600 mg/h and thus 25-30mg/kg BW/h (in a pig wighing 20 kg). This is also higher than what is reported elsewhere[55].

The animals were ventilated at a rate of 25 breaths per min and at tidal volumes of 10 ml/kg BW regardless of acid-base changes. This equals a minute volume of 5000 ml in a 20 kg pig, which is about 33% lower than the 7500 ml suggested in Flecknell's Laboratory Animal Anesthesia for a pig of this BW[53]. This volume was chosen to avoid excessive respiratory compensation of the expected acidosis resulting from the HS. In paper V, VO_2 was calculated by means of indirect calorimetry using a gas exchange and metabolic monitor. This integrated facility of the Elvira ventilator enables continuous measurement of VO_2 by calculating the difference between inspired and expired oxygen.

Hemorrhage model and estimations of blood volume

In experimental research on HS, two basic models are at hand. One is isovolumetric and involves the withdrawal of a specified blood volume based on BW. The other is isobaric and was first described by Wiggers and is therefore often called the Wiggers model[56]. In the latter model, animals are bled to a certain predetermined blood pressure. We chose the former model because it seemed more realistic when compared to the latter, although both of them have obvious drawbacks in simulating a real-life hemorrhage. Experimental hemorrhage can also be controlled or uncontrolled. In this project, we chose controlled hemorrhage to obtain data that are comparable and would depend on the variation of one variable only, namely core temperature.

Pigs may, however, contract their spleen to a certain degree in response to hemorrhage, which has caused some authors to splenectomize their pigs some time prior to the experiments[57]. This seems, however, to induce increased heart frequency in response to hemorrhage, compared to non-splenectomized controls. The spleen is therefore often retained in studies based on porcine models. The fact that the total body mass of red blood cells, corrected for BW, is similar to that of man supports this notion, although splenic contraction may contribute up to 20-25% of the red cell volume in pigs[58]. Furthermore, it has been shown that the total circulating blood volume is virtually the same in splenectomized and non-splenectomized pigs[57]. For these reasons, our animals were not splenectomized before the

experiments. Furthermore, a surgical event before the main experiments would constitute a confounding factor.

Before exsanguination began, the total blood volume was estimated to be 65 ml/kg BW[57]. In papers IV-V, the change in blood volume during the experiments was estimated by calculations of the blood hemoglobin concentration[59].

Papers I, II, IV: After preparation, 30 min were allowed for stabilization. Then baseline values were recorded and 50% of the individually calculated blood volume was withdrawn at a constant rate in 20-25 min using a roller pump. In order to prevent clotting of the roller pump, 1000 U of heparin was given i.v. immediately before exsanguination.

Paper V: When the animals were deemed to be in steady state after 30 min of rest and baseline values were noted, 40% of the individually calculated blood volume was withdrawn in 3-5 min by syringe aspiration without previous administration of heparin. In this study, the extraction of circulating blood amounted to only 40%, because the withdrawal was completed in only 3-5 min, which results in a greater sympatho-adrenal and hemodynamic response. This change in the HS- model stemmed from a desire to more closely imitate the hemodynamic insult created by uncontrolled hemorrhage in an aortotomy model in swine[60].
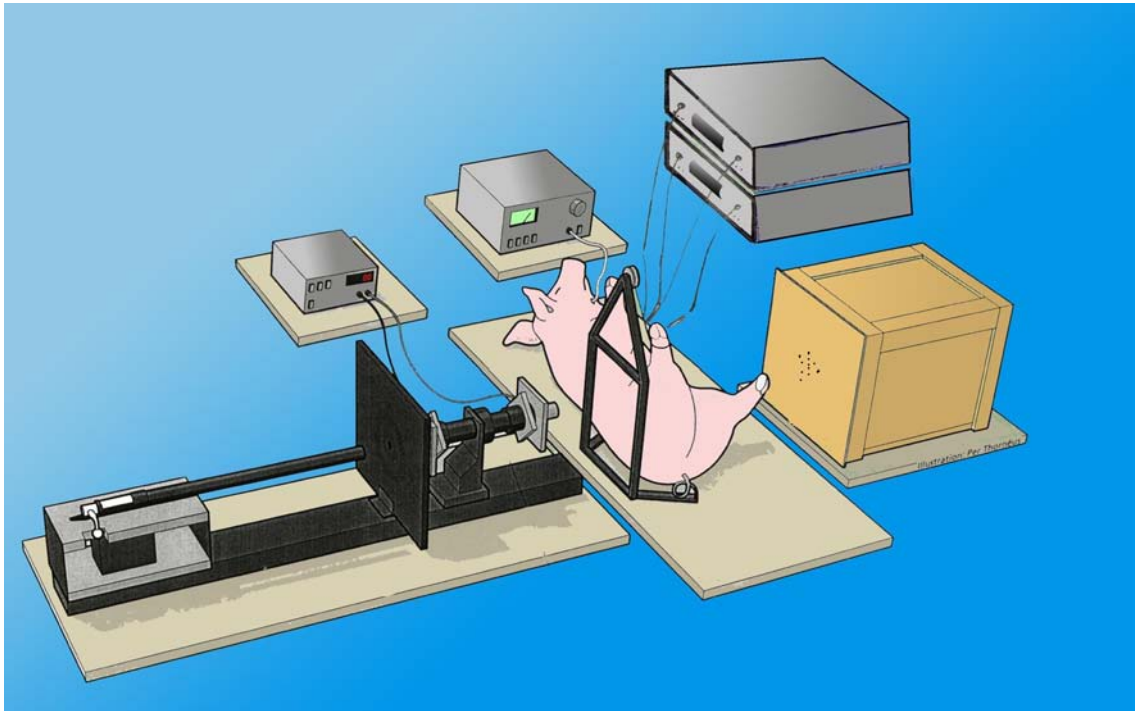
Fig. 1 The Swedish Missile Trauma Model

Trauma model studies (papers III and IV)

To achieve a significant and standardized soft-tissue injury, the Swedish Missile Trauma Model was used[61]. With this trauma model, a well-directed and reproducible injury can be inflicted while minimizing the risk of collateral injuries. When using this very model on pigs of similar size, Riddez and coworkers estimated the total blood loss from the injury in NT animals to be 22 ± 3 ml (unpublished data). The point of aim of the weapon was the posterolateral aspect of the right suspended thigh (Fig. **III:2**). The exit velocity of the projectile was set at 1500 m/s. Since the muzzle-to-target distance was only about 60 centimeters, it is safe to assume that the muzzle and impact velocities were nearly the same.

In paper IV, exsanguination began within a min of the injury.

Hemodynamic measurements and blood sampling

The left external jugular vein was catheterized (Portex Ltd., Hythe, Kent, England) for infusion of ketamine after a paramedian incision on the neck. Through the same incision, a catheter was introduced into the left common carotid artery for blood sampling, recording of arterial pressures and exsanguination. A right paramedian neck incision was performed in order to introduce a flow-directed, multichannel thermodilution catheter (CritiCath, SP5105H, size 5F, Spectramed, Inc., Oxnard, CA (**I-IV**), Swan-Ganz, Edward labs, Santa Ana, CA (**V**)) into the pulmonary artery by way of the right external jugular vein. The accurate position of

the catheter was determined by repeated measurements of the pulmonary capillary wedge pressure, after which registrations of MPAP, CO and SvO2 (**V**) were performed. The catheters in the left common carotid artery and the pulmonary artery were connected to a Sirecust 1280 (Siemens Medical Systems, Inc., Danvers, MA) for continuous pressure monitoring. The MPAP was measured via the pulmonary artery catheter, the tip of which was located in the pulmonary artery. The CVP was measured in the right atrium through a side-hole in the catheter. CO was estimated with thermodilution by rapid injection in triplicate of 3 ml of saline through the catheter, the injection port being 5 cm from the tip. The temperature of the injectate was similar to that of the ambient temperature. In cases where the difference between these two temperatures was 8°C or less, cooled saline with temperatures of 2-5°C was used as an injectate instead. CO was determined using an Oximetrix 3 computer system (Abbott Critical Care, Abbott Laboratories, North Chicago, IL) and was defined as the average of the three measurements. The thermodilution method has been found to be valid for use even during HT[62].

Samples of arterial blood for analyses of blood gases, Hb, and electrolytes were extracted through the arterial line and measured using a BGE analysator and CO-Oximeter 482 (Instrumentation Laboratory Srl, Milan, Italy) (**I-III**) or a Gem Premier Plus (Instrumentation Laboratory Srl, Milan, Italy) (**IV-V**). We refrained from correcting arterial blood gases for core temperature and analyzed them at 37°C. Since neutral pH rises with cooling, so should the blood pH. This approach ensures intracellular electrochemical neutrality, which optimizes enzyme and protein function by maintaining a degree of relative alkalinity in the blood. Hence, it appears that the HT patient is metabolically most stable at slight alkalosis[24, 27, 63].The increase in arterial PO_2 in HT animals in the present study results from this strategy. This increase will also affect the calculations of arterial oxygen content and DO_2, but the effect is so small relative to the other factors in the calculation that there are no detectable differences between the groups.

CO and the oxygen content of arterial (CaO_2) and venous blood (CvO_2) were used to calculate DO_2 and VO_2 as given in the definitions. However, VO_2 in paper IV was estimated by the ventilator through indirect calorimetry, in which an analysis of expired air allows the determination of VO_2 and the production of CO_2.

Counts of blood cells and analyses of lactate, lactate dehydrogenase, blood glucose, creatine kinase and creatine kinase (muscle/brain) were performed using standard clinical kits. Plasma concentrations of catecholamines and IL-6 were measured using high-pressure liquid chromatography[64] and a bioassay using a B-cell hybridoma cell line[65], respectively.

Thromboelastography was used in paper V to evaluate the effects of HT on coagulation and fibrinolysis[66].

Hypothermia protocol, studies I-IV

The HT bed (Auto Hypotherm, Heljestrands, Sweden) was developed for use in patients with neurosurgical conditions. It looks and works like a big incubator, allowing circulation of air at any given temperature around the subject placed in it and under the plexiglass hood. The HT bed was chosen in this project for practical reasons and because it has been in clinical use[67].

 Animal preparation took place on the HT bed, minimizing the risk of technical disturbances while moving the anesthetized, catheterized, and intubated animal. All pigs were covered with a blanket to maintain their normal core temperature (38.5-39.5°C), which was determined with the aid of the pulmonary artery catheter. After inflicting the trauma (HS and/or GSW), randomization took place. Animals randomized to NT retained the blanket, while animals randomized to HT were immediately relieved of the blanket, whereupon the plexiglass hood was put in place to cover the animals. The ambient air temperature in the HT bed was lowered to about 4°C within minutes after starting the bed's compressor. Small windows in the plexiglass allowed tubes and catheters to exit the animal without interruption. Active cooling was terminated at 31-32°C to allow some afterdrop to the target core temperature of 30°C. At this point, the hoods were removed and the animal were thus exposed to the ambient air with temperatures at 23-25°C. The target core temperature of 30°C was chosen to achieve a significant reduction of metabolism while avoiding such complications of HT as cardiac arrhythmias.

Pigs normally have a core temperature of 38.5-39.5°C. The exact definition of porcine HT remains to be established. In this thesis, it has been assumed to be similar to that of humans since the physiology of these two species is in many aspects very much alike.

It is sometimes questioned what the true thermal core is, but it is now commonly accepted that the temperature in the hypothalamus constitutes this entity[20]. For this reason, some authors employ tympanic temperature recordings. We used the temperature probe in the tip of the pulmonary artery catheter for recordings of the core temperature. The deviations of the temperatures in the heart and lungs from that of the brain are known to be so miniscule that they have no practical implications. For instance, Marion et al. reported that in 95% of

over 4000 simultaneous measurements, rectal and brain temperatures did not vary by more than 0.5°C[40].
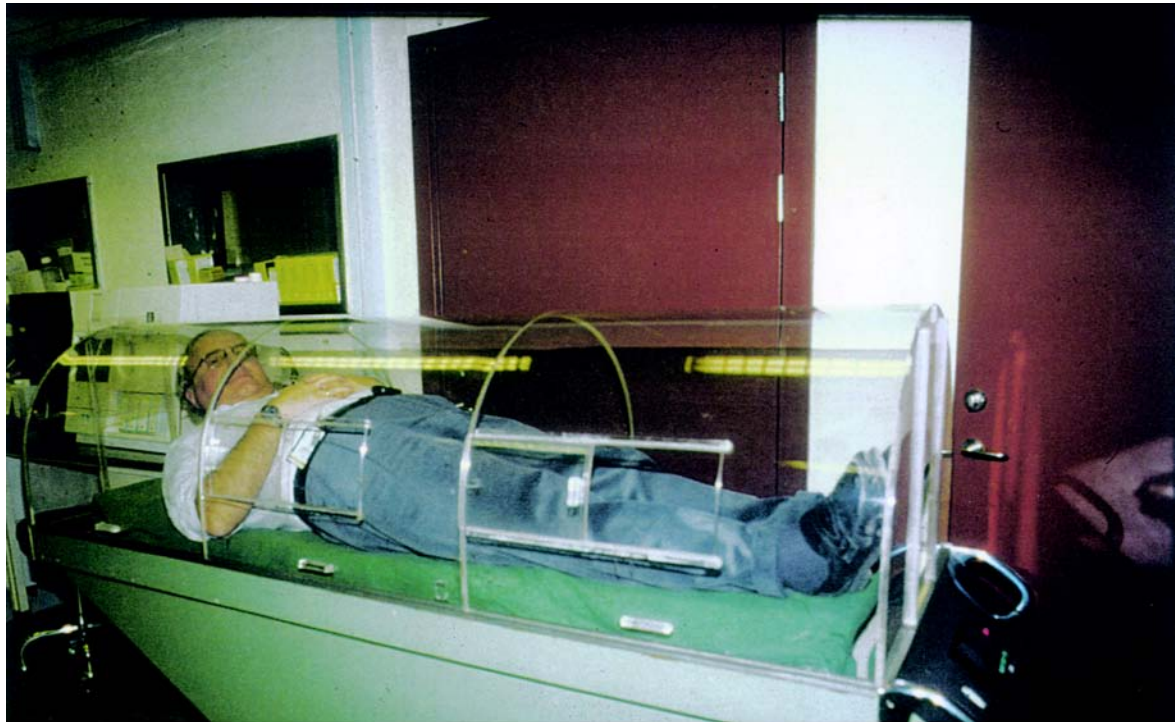

Fig. 2 The Hypothermic bed

Hypothermia and rewarming protocol, paper V

In study V, HT was induced as described above, but cooling was stopped when the core temperature reached 33.5°C since the target temperature in this study was 32°C. The reason for increasing the target core temperature to 32°C in this study was to evaluate whether or not the possibly protective metabolic alterations found at 30°C would be maintained with a wider margin from core temperatures at which various complications have been described.

Rewarming was initiated after 30 min at the target temperature and was achieved by instilling Ringer's acetate in the urinary bladder and simultaneous circulation of warm air (42°C) in the HT bed. The exchange of fluid in the bladder was maintained at a rate to keep the vesical temperature at 42°C. Warm Ringer's acetate (<42°C) was also given i.v. After regaining the baseline core temperature, the animals were observed for another two hours.

The HT bed was used for rewarming for practical reasons and because it allowed the combination of active core and topical rewarming.

<u>Controls</u>

To specifically study the metabolic effects of shivering in this model, five pigs were exposed to our usual preparation and, subsequently, a standardized GSW, as described above, with similar energy transmission levels. HT was induced in all of them, and in addition to the continuous infusion of ketamine, the animals were given repeated i.v. injections of pancuronium for muscle relaxation. The animals were followed for 4 h after the injury.

To isolate the effects of HT and the preparation and handling of the animals, 10 pigs were randomized to HT or NT, but they were not exposed to any other trauma. The animals were followed for 4 h.

Table 1. Study design, papers I-V

I & II.	Surgical prep	Steady state	Baseline	Hemorrhage	HT induction		End of experiment
Time		30 min	–30 min	–30 to -5 min	0 min		240 min
III.	Surgical prep	Steady state	Baseline	GSW & HT induction			End of experiment
Time		30 min	0 min	10 min			360 min
IV.	Surgical prep	Steady state	Baseline	GSW	Hemorrhage		End of experiment
Time		30 min	–30 min	–20 min	–20 to 5 min		240 min
V.	Surgical prep	Steady state	Baseline	Hemorrhage	HT induction	Rewarming	End of experiment
Time		30 min	0 min	0-5 min	5 min	100 min	420 min

Note: Hemorrhage in all relevant studies was completed at or near time 0 min, at which point HT was induced.

<u>Statistics</u>

Results are expressed as the mean and standard error of the mean (SEM). Repeated-measures analysis of variance (ANOVA) was used for statistical evaluations within each group and a two-way ANOVA was employed for comparisons between groups. Differences were considered statistically significant if $P<0.05$.

In paper I, the Wilcoxon matched-pairs test was used to evaluate differences within each group and the Wilcoxon rank-sum test was used for comparisons between the groups.

When appropriate, Student's paired t-test was performed as well.

PRINCIPAL RESULTS

I.   **Acute Metabolic and Endocrine Effects of Induced Hypothermia in Hemorrhagic Shock: An Experimental Study in the Pig.**

Withdrawal of 50% of the individually calculated blood volume in anesthetized piglets during 25 min resulted in a persistent increase of plasma catecholamine levels in the NT controls (Fig. **I**:2,3). In HT animals, this increase was transient and plasma adrenaline levels decreased faster than with plasma noradrenaline, eventually going below baseline levels. Simultaneously, serum potassium levels increased gradually in the controls, but decreased transiently in the HT group (Fig. **I**:4). PO_2 increased by 55% (mean) in the HT group but was largely unchanged in the controls. Levels of pH decreased similarly within normal limits regardless of the core temperature. Plasma levels of lactate more than doubled in both groups in course of the experiment.

II.   **Acute Hemodynamic Effects of Induced Hypothermia in Hemorrhagic Shock: an Experimental Study in the Pig.**

A reduction of the circulating blood volume by 50% in anesthetized piglets during 25 min resulted in a prompt increase in HR and a simultaneous reduction of CO, CI, SV, MAP, and VO_2 in both groups. In the NT controls, these changes were persistent. With the induction of HT, however, HR decreased to levels below baseline (Fig. **II**:2). MAP and CO also continued to decrease in HT animals, while SV was not affected by cooling (Fig. **II**:3-5). DO_2 in both groups decreased after exsanguination, but was further reduced with cooling (Fig. **II**:6). The systemic vascular resistance increased in both groups after the hemorrhage, but gradually returned to baseline levels irrespective of the core temperature.

The total blood leukocyte count and polymorphonuclear leukocyte count increased in both groups in response to the hemorrhage, but decreased gradually in HT animals one hour and onwards after the hemorrhage (Fig. **II**:7). The platelet count decreased in both groups, but only significantly so with cooling. The Hb decreased significantly after the hemorrhage by a mean of 26% in the HT group and 20% in the controls. This difference between the groups was not significant.

Three NT animals succumbed to HS, while all HT pigs survived.

III.   Effects of Induced Hypothermia after Severe Soft-Tissue Injury

Infliction of a standardized soft-tissue injury in anesthetized piglets followed by cooling to a core temperature of 30°C caused a reduction of HR and MAP (Fig. **III**:3, 4). In the controls, these parameters decreased as well but not to the same extent creating significant differences between the groups. In contrast, CI and SV decreased similarly regardless of core temperature (Fig. **III**:5). There were no significant changes in either group regarding CVP, MPAP, PCWP, and the systemic and pulmonary vascular resistances.

Serum potassium levels decreased transiently with HT, but *increased* by approximately 34% (mean) in the controls (Fig. **III**:6). The difference between the groups was significant.

The serum activity of creatine kinase and lactate dehydrogenase increased in both groups after the GSW, but more so in HT animals (Fig. **III**:7, 8). Serum levels of lactate increased transiently in both groups after the insult.

Plasma levels of adrenaline and noradrenaline decreased in both groups over time, but this was more pronounced for both hormones with cooling (Fig. **III**:9, 10).

DO_2 decreased gradually and similarly in both groups (Fig. **III**:13).

A reduction of blood leukocyte counts was found with HT, while the leukocyte counts increased with normal core temperature (Fig. **III**:14). In contrast, the counts of polymorphonuclear leukocytes (PMN) was not affected by HT, but had increased by 61% (mean) in the controls two hours after the GSW. Platelet counts gradually decreased with cooling, but were unaltered in NT animals. The Hb fluctuated around the baseline level in the HT group, whereas a gradual decrease occurred in the controls (Fig. **III**:15). The difference between the groups was statistically significant for counts of leukocytes and PMN.

There was no mortality in either group.

IV.   Induced Hypothermia after High-Energy Soft-Tissue Trauma and Subsequent Hemorrhagic Shock

A standardized soft-tissue injury in anesthetized piglets and immediately subsequent withdrawal of 50% of the blood volume in 25 min followed by either HT or NT resulted in a reduction of CO and MAP by approximately 50% in both groups (Figs. **IV**:2-3). HT markedly reduced the VO_2, but did not affect DO_2 as compared to the controls (Fig. **IV**:5-6). In NT

animals, VO_2 decreased slightly during the hemorrhage, but baseline levels were attained within two hours. As a result, the ER increased from approximately 35% in both groups to about 75% after the insults (Fig. **IV**:7). With the HT-induced reduction of VO_2, ER decreased to 50%, while it remained high in the controls. HR and plasma catecholamines increased in both groups as a result of the insults, but eventually returned to near baseline levels in the course of HT. CO, CI, and SV all decreased dramatically in both groups without any differences. The CVP and MPAP decreased in response to the insults and remained low in both groups. The systemic vascular resistance increased transiently after the insults in the controls and then returned to baseline levels. In animals exposed to cooling, this resistance continued to increase reaching a maximum nearly 50% higher than in the controls two hours after the insults.

The insults caused a reduction of arterial BE and pH within normal limits, core temperature notwithstanding. Arterial PO_2 and SaO_2 were largely unaffected in the controls, but increased with cooling. Levels of pH decreased within normal limits in both groups. Plasma levels of lactate more than doubled in both groups after the insults. There were no significant differences between the groups.

Levels of both serum potassium and creatinine both increased in HT animals and controls alike after the insults (Figs. **IV**:9,10). Cooling caused a decrease in both parameters. In NT animals, however, both serum potassium and creatinine increased to levels approximately 30% above that of HT pigs, reaching the upper echelon of normality.

At the end of the study, the calculated blood volume was 44% of baseline values in the controls. The corresponding figure in the HT group was 47%. Plasma levels of IL-6 were significantly lower in HT animals at the end of the experiment. Hb decreased by about 16% in both groups during the course of the study. Blood leukocyte counts decreased similarly in both groups after the insults. One hour into the study, counts of leukocytes started increasing in the controls while they decreased in the HT animals. The counts of polymorphonuclear leukocytes mimicked that of the total leukocyte counts, as did monocyte counts (Fig. **IV**:11). Platelet counts decreased gradually and similarly in both groups, but the decrease was significant in HT animals only.

All HT animals survived, but one NT pig died of HS.

V.   Induced Hypothermia and Rewarming after Hemorrhagic Shock

Withdrawal of 40% of the individually calculated blood volume in anesthetized piglets within a few minutes, followed by fluid resuscitation and either HT or NT, and, eventually, rewarming in the HT group resulted in the depression of MAP, CI and SV in both groups (Figs. $\mathbf{V}$:3,4,5). In both groups, DO_2 decreased in response to the exsanguination, increased during resuscitation and decreased again during the rewarming phase. In contrast, VO_2 decreased significantly more in HT animals, causing the ER to decrease significantly below levels in the controls (Fig. $\mathbf{V}$:6). With rewarming, the two groups eventually regained similar levels of VO_2 and ER. Serum potassium levels increased transiently in both groups after the hemorrhage (Fig. $\mathbf{V}$:7). With cooling, serum potassium decreased but increased again during rewarming to reach hyperkalemic levels at the end of the experiment. In the controls, serum potassium levels increased continuously, reaching nearly the same levels as those in HT animals.

HT induced a slight prolongation of the time until clot formation started and the time needed for formation of the clot (Fig. $\mathbf{V}$:8). These changes were reversed during rewarming. Clot formation was not affected in the NT group. The strength of the fibrin clot was not affected by HT, but some values were reduced among the controls (Fig. $\mathbf{V}$:9). The only significant difference found between the groups was that of clot lysis, which was significantly reduced during HT but remained unaffected in the NT group (Fig. $\mathbf{V}$:10; $P<0.05$).

The blood platelet count decreased gradually by 22% (mean) in the HT animals and by 31% in the controls during the course of the experiment, but the difference was not significant.

One HT pig and three of the controls died during the experiment.

GENERAL DISCUSSION

Plasma catecholamines and serum potassium

In man and most of the common experimental model animals, the most conspicuous hormonal response to hemorrhage appears to be a considerable increase in the secretion of plasma catecholamines from the adrenal medulla[51, 68, 69]. These hormones are known to lower levels of plasma potassium[70, 71].

When these beings are exposed to HT there is instead no uniformity in the hormonal response. Consequently, when these two insults are superimposed on each other, the response is likely to vary. Furthermore, the hormonal response will vary depending on any anesthetics being used, the degree, type, and longevity of the HT, and, possibly, the species.

It is possible that when HT is superimposed on HS, as in paper I, the catecholamine response, which, as far as *plasma* catecholamines are concerned, is mainly modulated by the adrenal medulla, has already been exhausted. This would explain why there is only a small increase, soon followed by a decrease, of plasma catecholamines when surface HT is induced. The decrease is found immediately after the induction of HT in study IV. This consistent decrease may be due, not only to exhausted catecholamine reserves but also to gradual downregulation of the metabolism in the adrenal medulla (studies I and IV). It is noteworthy that plasma levels of noradrenaline were approximately three times higher 30 min after the termination of the hemorrhage in study I as compared to plasma levels of this hormone in study IV at the same point in time. Plasma concentrations of adrenaline were, however, comparable in both of these studies. There are no obvious explanations for this disparity in plasma noradrenaline response.

In study III, the induction of HT following the gunshot only caused a slight increase in the plasma catecholamine levels (**III**:9, 10), but they decreased with time. These findings conform with those of Derek Maclean and Donald Emslie-Smith, who describe a sharp decline in the medullary output of both catecholamines after the initial surge when HT is induced[23].

In studies I, III and IV it was consistently found that plasma adrenaline levels decreased before levels of noradrenaline. Furthermore, the decrease in plasma adrenaline appeared to correlate quite well with the decrease in core temperature (I, III, IV). AC Guyton notes that approximately 80% of the secretion from the adrenal medulla is adrenaline and 20% is noradrenaline. However, he also reports that the relative proportions of these catecholamines

"change considerably under different physiological conditions"[72]. This variability is well in line with our findings, where the increase in plasma noradrenaline was most pronounced in study I and that of adrenaline was most pronounced in studies III and IV. In contrast, a 30% hemorrhage at a rate of 1% blood volume per minute in a series of 104 conscious swine (BW 20 kg) caused an increase of over 300% (mean) of plasma adrenaline and of nearly 200% of plasma noradrenaline levels[73]. Moreover, it is possible that the use of ketamine anesthesia in HS further stimulates the sympathetic nervous system and causes an added increase of plasma catecholamines[74]. Hence, the explanation for the discrepancy found in plasma catecholamine response in our studies is multifactorial, but it is clear that, with our model, HT results in a significant reduction of plasma adrenaline and noradrenaline alike. This reduction explains some of the cardiovascular effects noted in this thesis and it may save the myocardium from metabolic exhaustion[75].

Potassium homeostasis is under the influence of many forces. Normally, the acute regulation of extracellular potassium levels is achieved by alterations in the distribution of potassium between the extracellular and intracellular fluids. The movement of potassium is largely under hormonal control. Hence, insulin, catecholamines, and aldosterone promote cellular uptake of potassium. This occurs mainly through activation of the sodium-potassium pump mechanism which creates a high intracellular potassium concentration.

Levels of serum potassium appear to vary in HS. In dogs, serum potassium has been reported to increase following hemorrhage[76]. Hannon and Bossone reported a small, but significant, reduction in plasma potassium levels from 4.5 to 3.7 mmol/L in conscious pigs subjected to 50% blood loss during 60 min[77]. Moreover, pigs subjected to isobaric hypovolemia often show electrolyte changes during hypotension that are just the opposite to those seen during fixed-volume hemorrhage. The potassium increase may be due to deterioration of sodium/potassium pump activity[51].

HT, in turn, also seems to have a variable impact on serum potassium levels, but Maclean and Emslie-Smith have concluded that there is a lack of convincing evidence in man that HT by itself significantly alters the serum potassium concentration[23]. In contrast, Boelhouwer and coworkers showed a significant correlation between the decrease in serum potassium and the fall in rectal temperature[78].

Destruction of skeletal muscle causes a leakage of substances such as potassium into intravascular and extracellular spaces, which probably influenced serum potassium levels (studies III and IV).

With regard to the above, it is obvious that in our experimental settings a number of forces acted simultaneously to increase and decrease serum potassium levels. In the absence of tissue destruction, HT caused a transient reduction of serum potassium (paper I). In the NT controls, serum potassium levels increased gradually, reaching hyperkalemic levels after bleeding. The one pig that died of HS in this study had a terminal serum potassium concentration of 6.9 mmol/L. These observations conform with data from a study by Johnson et al. on rats in HS in which a temperature-dependent rate of rise in plasma potassium was demonstrated, suggesting that HT applied late in the compensatory phase would slow the loss of cellular potassium[79].

In studies III and IV, the GSW to the right hindlimb caused significant destruction of muscle tissue. This made the serum potassium concentration increase significantly in the controls, whereas a transient, but significant, reduction was noted in HT animals after the GSW (Fig. **III**:6, **IV**:9). In paper V, a similar reaction was found although no trauma was inflicted on the animals (Fig. **V**:7). However, during the extended experiment described in paper V, serum potassium levels in the HT group not only returned to baseline levels but eventually increased to reach hyperkalemic levels comparable to those in the controls. This suggests that in HS with or without soft-tissue trauma, HT has a transiently stabilizing effect on serum potassium.

In study IV, it appeared that serum potassium levels were inversely correlated with those of plasma adrenaline. Accordingly, plasma adrenaline levels increased markedly in response to the GSW and hemorrhage in this paper. The tissue destruction and local ischemia secondary to the GSW explains the initial increase of serum potassium levels in spite of elevated plasma adrenaline concentrations. With HT, plasma adrenaline levels slowly decreased and, 2 h into cooling, baseline levels were reached. Simultaneously, the seemingly depressant effect of plasma adrenaline on serum potassium appeared to be extinguished as levels of this electrolyte in serum started to increase again. The persistently high levels of *both* plasma catecholamines and serum potassium in the NT controls indicate that another mechanism as well was at work elevating serum concentrations of this electrolyte.

In studies II and III, the adrenaline-potassium correlation was similar. Initially, elevated plasma adrenaline levels caused a quick reduction of serum potassium. In the course of HT, plasma adrenaline was slowly reduced and, concomitantly, serum plasma increased. In study V, however, hypokalemia was supplanted by gradual hyperkalemia during the course of normalization of the core temperature. The lack of data on plasma catecholamines in this

paper, makes it impossible to draw conclusions about the relationship between these hormones and serum potassium impossible regarding this paper.

When 10 animals where randomized to either HT (n=5) or NT (n=5) after our standard preparation, serum potassium levels decreased with cooling, while they increased somewhat in the controls. However, the changes in both groups were within normal limits (unpublished data). There is no information on plasma catecholamines from this series of experiments either.

In summary, of all the driving forces that may affect serum potassium levels in our experimental settings, plasma adrenaline appears to be of major importance in cases of HT. Plasma levels of this hormone depend on the core temperature. Consequently, when HT is firmly established, plasma adrenaline levels will decrease and so will the depressive effect of this hormone on serum potassium levels. Our data corroborate findings in previous studies on the effect of HT on serum potassium and show that this effect is transient and temperature-dependent. The persistently high levels of both plasma catecholamines and serum potassium in the controls suggest, however, that the flow of potassium in these studies is under the influence of another driving force as well. There are no data in the present study to determine whether this force is, in fact, the combined forces of reduced renal clearance due to HS reducing renal circulation and the glomerular filtration rate, the shock itself with concomitant disturbance of the microcirculation or tissue distruction due to the GSW (papers III and IV), or only one of these forces. Considering that the acid-base status in the present papers remained fairly unaffected, this would seem to be an unlikely explanatory mechanism.

Hemodynamics and metabolism

By virtue of the significant hemorrhage in studies I, II, and IV, all animals were in decompensated shock as no fluid resuscitation was carried out in these studies. Only standard fluid maintenance comprising 10 ml/kg Ringer's acetate was provided throughout the experiments. In contrast, pigs in study V were resuscitated with Ringer's acetate in a volume of three times the volume of withdrawn blood. In addition to that, the exsanguination in study V occurred much faster (3-5 min vs. 25 min) and, after the fluid resuscitation, the animals were given Ringer's acetate at the above-mentioned maintenance rate. Standard fluid resuscitation was carried out 10 min after the hemorrhage to imitate a clinical situation.

There were no laboratory signs suggestive of splenic contraction secondary to the hemorrhage in any of the studies. The hematocrit and Hb levels decreased uniformly in both

groups after the hemorrhage. Leukocyte counts increased, however, during the first hour after the exsanguination, but this is more likely an effect of catecholamine-induced recruitment from the marginal pool[80].

HT pigs seemed to compensate for the diminished circulating blood volume with less vigor, which probably was due to diminished metabolic demands, indicated by the marked decrease in VO_2 in this group. The hemodynamic compensatory mechanism seemed to be particularly weak in HT animals exposed to hemorrhage only (study II). Thus, in the HT group, MAP and CO decreased significantly below the levels in the controls (Fig. **II**:3-4), while SV did not differ from that in the NT animals. Also, CO in the HT animals described in this paper seemed to follow the decrease in core temperature rather closely. In contrast, MAP, CO, SVR, and DO_2 were strikingly similar in both NT and HT animals when the hemorrhage was preceded by a high-energy GSW (study IV). Compared to the hemodynamics described in paper II, the attendant soft-tissue trauma in paper IV produced remarkably few effects on the central hemodynamics. These findings are in sharp contrast to those reported by Rady et al.[81]. In Rady's study, skeletal muscle injury in both hind legs of similarly sized pigs followed by a 40% blood volume extraction caused a reduction of tolerance to hemorrhage as compared to hemorrhage alone. This discrepancy may be explained by differences in study design. Accordingly, as outlined in paper IV, Rady et al. ventilated the pigs with isoflurane, which is a potent vasodilator. Furthermore, the animals in that study had both femoral arteries ligated, which could induce considerable ischemia and secondary cardiovascular depression. At any rate, the differences warrant further investigation of how soft-tissue trauma modulates HS.

The hemorrhage in studies I and IV caused a reduction of pH within normal limits in both groups, while plasma lactate increased markedly after the hemorrhage regardless of the core temperature. These results suggest that the hemorrhage induced lactic acidosis, which was not reduced by metabolism in the liver, presumably because of shock-induced malfunction of this organ. The data also suggest that the ventilation of the animals was efficient. The lack of differences between the groups indicate that HT does not deteriorate the metabolic situation. There is also no basis for concluding from these data that the induction of HT either ameliorates or deteriorates the metabolic imbalance induced by the shock. In contrast, the reduction of VO_2 and the resultant decrease in ER in study IV constitute indirect evidence that tissue oxygenation in HS is enhanced by HT despite the shivering. In studies II to V, DO_2 in HT pigs decreased similarly (studies III to V) or somewhat more (paper II) than in the controls. Although there is no data on tissue oxygenation, this suggests that the left shift

of the oxyhemoglobin dissociation curve only has a small effect on oxygen delivery in this experimental setting. Gutierrez and coworkers arrived at the same conclusion when studying the effects of HT on oxygenation in dogs subjected to hypoxic hypoxia[82]. In a review of the pathophysiology of HT, James B. Reuler explained that the left shift of the oxyhemoglobin dissociation curve is not clinically relevant because of the concomitant decrease in oxygen requirements[26]. Furthermore, in studies IV and V, HT caused a significant reduction of VO_2. In study V, this reduction coincided well with both the fall and rise of the core temperature.

The GSW alone, followed by HT, caused significant reductions of HR and MAP, but no further decrease in CI, SV, and DO_2 compared to the NT controls (study III). Moreover, in a series of animals (n=10) exposed to nothing but the experimental preparation and then randomized to either HT (n=5) or NT (n=5), the most conspicuous hemodynamic event was the decrease in CI, which was 47% (mean) in HT animals while it increased by 14% (mean) in the controls (unpublished data). These changes in HT pigs are well explained by a decrease over time in both HR and SV.

In study V, the hemodynamic influence of HT was small, probably due to the concomitant fluid resuscitation. Hence, two hours into this study when the animals' blood volume was replenished, rewarming was started. The absence of deleterious effects of HT in this setting is nevertheless noteworthy with regard to the current dogma on HT in shock and trauma.

To summarize the hemodynamic changes found in the present studies, HT, *per se*, appeared to depress most hemodynamic parameters, except in study V. When two insults (GSW and hemorrhage) were inflicted consecutively within minutes of each other, this depressive effect was not equally apparent except for HR, which decreased, and SVR, which increased as compared to the controls. This surprising finding is difficult to explain and warrants further investigation. However, it is conceivable that the combined insults described in study IV had such a profound depressing impact on the cardiovascular function of the animals that the depressive effect of HT *per se* was concealed. Furthermore, the animals in study I responded with higher catecholamine levels than those in study IV, as mentioned previously, which makes the cardiovascular effects in these papers even more surprising and bewildering. Accordingly, after two hours, CO in the HT animals in study III (Fig. **III**:5) was approximately 3.5 L/min (mean), while, in study IV, it was not even half of that and, in study II, it was even lower (Table 2). It is indeed remarkable that the HT animals in studies II and IV managed to survive with such a low CO for hours on end.

The ultimate end-point in studies on HS and trauma is mortality. As indicated in Table 2, only one HT animal died despite the severely compromised hemodynamics in this group, while, altogether, seven of the NT controls succumbed to HS. Although the numbers may be too small to allow reliable statistical inferences and the study protocols differ somewhat, they are suggestive of a protective effect induced by HT. According to Fisher's exact one-tailed test, the difference in mortality between all HT and NT animals in papers I-V is significant (P=0.03).

Table 2. Two h into the studies, the target temperature was usually reached and the following parameters were altered as described below (means). Data on overall death are also noted.

	HR		CO		MAP		SV		Death	
	(bpm)		(L/min)		(mmHg)		(ml/beat)			
	HT	NT	HT	NT	HT	NT	HT	NT	HT	NT
Papers I & II (hemorrhage)	104	169	0,9	2	38	56	9,7	11	0	3
Paper III (GSW)	93	109	3,5	4	55	74	38	37	0	0
Paper IV (hemorrhage & GSW)	138	165	1,6	2,2	60	60	12	14	0	1
Paper V (rapid hemorrhage)	96	98	3,3	2,6	64	60	36	26	1	3
Controls (HT vs NT)	104	87	2,3	2,5	87	84	21	29	0	0

Blood cells, coagulation and immunocompetence

As mentioned previously, induction of HT is known to cause both thrombocytopenia and leukopenia through sequestration of these blood cells in the spleen and in the bone marrow. In studies II-IV, HT universally caused a reduction of blood platelets. Although this decrease was not statistically significant as compared to the controls, there was an obvious trend. Furthermore, leukocytes decreased consistently and significantly in studies II to IV after the initial increase, which is a physiological catecholamine-induced response to the hemorrhage. In study V, platelets decreased significantly and similarly in both groups, while blood leukocytes fluctuated around baseline levels in both groups.

Clinically, the HT-induced changes in blood cell counts found in studies I-IV may materialize as coagulopathy and increased susceptibility to infections, but the available data are inconsistent. As indicated above, some reports suggest that HT in patients undergoing surgery causes increased bleeding[38, 83]. In contrast, Marion et al. failed to detect increased

bleeding in patients subjected to induced HT after traumatic brain injury[40]. Furthermore, Johansson et al. did not find any differences in bleeding between NT and mildly HT patients operated on for total hip replacement[39]. In studies I-V, the platelet counts decreased significantly in animals exposed to cooling, but the decrease was not significant in the controls and the difference between the groups was not significant either, as mentioned above. The animals were subjected to the trauma of the surgical preparation and/or the GSW. Some of the animals in studies I and II also underwent a thoracotomy. There were no signs of bleeding from the wounds in any animal.

The ultimate strength of the clot in study V was not affected by HT, which is in line with data on patients undergoing liver transplantation and coronary revascularization[84, 85]. However, Watts *et al.* found a significant reduction of clot strength in trauma patients with core temperatures below 34°C. The strength of blood clots depends on the interaction of platelets and fibrin, and severe and uncontrolled non-surgical trauma possibly alters the quality and quantity of these two factors unfavorably. Fibrinolytic activity also seemed to be slowed down by HT, suggesting a temperature-dependant inhibition of fibrinolytic enzymes. The fact that few significant differences between the groups in TEG-related data was found is in itself interesting, but must be interpreted cautiously. Overall, the TEG results in study V suggest that induction of HT in trauma patients may not promote rebleeding once the clot is formed, but if rebleeding occurs, hemorrhage may be more prolonged.

Development of adult respiratory syndrome (ARDS) and multiple organ failure are both due to an overreaching immunological response to injuries. The immunological effects of HT are not yet fully understood, which is indicated by the variability in results presented by different researchers. Some clinical investigations have demonstrated increased susceptibility to infections in surgical patients inadvertently exposed to HT[44]. Experimental data seem to corroborate this[86]. In contrast, Villar and Slutsky have demonstrated improved oxygen extraction and survival in patients with septic ARDS when treated with HT[49]. In study IV, but not in study III, the HT animals had significantly lower levels of plasma IL-6. This difference in IL-6 evolved slower than in blood leukocytes. Although Lin et al. and Biffl et al. have shown that IL-6 activates polymorphonuclear leukocytes and postpones their apoptosis[87, 88], the reduction of leukocytes in our studies (I-IV) is probably attributable primarily to sequestration of these cells in the bone marrow and reduced recruitment from the marginal pool because of decreasing catecholamine levels and not to lower levels of plasma IL-6 in HT animals. However, since plasma levels of IL-6 are proportional to the severity of the injury[87], our data would indicate that HT mitigated the early response to the injury. The HT-induced

reduction of blood leukocytes in our studies may also be due to trapping of these cells in, for example, the pulmonary vascular bed, which could instigate ARDS. If, however, further research shows this not to be the case, our findings could be an expression of HT mitigating the immunolgical response to injuries.

In summary, the present thesis provides some new views on the effects of induced HT in HS and/or soft tissue injury. Using the HT bed to induce HT is advantageous in that it is non-invasive. A major drawback with this method, however, is the the speed with which core cooling and rewarming can be achieved. Clinically, various forms of cardio-pulmonary bypass with a heat exchanger provides an expeditious means of controlling core temperature. The invasivity of this latter method will hardly constitute a problem if the method is ever used in trauma patients since they will have to be subjected to invasive monitoring and treatment at any rate.

Forthcoming studies in this field will have to determine not only the best means of controlling core temperature, but what the optimum core temperature in traumatized and/or hemorrhaged subjects is. This will likely occur around 33-34°C, where most of the beneficial effects of HT are in place but few of the detrimental ones.

With regard to our findings of reduced counts of various leukocytes in blood and also lower plasma IL-6 levels in HT animals, another area for future exploration is to further characterize the immunological implications of HT in HS and/or trauma.

CONCLUSIONS

♦ Induced moderate or mild HT does not increase mortality when imposed in pigs after HS, soft-tissue injury, or the combination of the two. To the contrary, induction of HT seems to reduce mortality in these studies despite severely compromised central hemodynamics. Induced HT appears to exert a protective effect after these traumatic insults by reducing the metabolic demands and thus creating a metabolically wider margin to cope with the reduced perfusion. These effects are reversed by elevating the core temperature to baseline levels. The exact mechanism behind the protective effect of induced HT remains to be identified.

♦ The hemorrhage causes a rapid increase in plasma catecholamines, which, in turn, depresses serum levels of potassium in the presence of HT. HT slowly reduces levels of plasma catecholamines, which exstinguishes the effects of these hormones on serum potassium. Serum levels of this electrolyte will then be subjected to the same elevating driving force that influences the serum levels of potassium in NT controls.

♦ The increase in plasma catecholamines mobilizes blood leukocytes from the marginal pool. With cooling, plasma levels of catecholamines will diminish and so will the counts of blood leukocytes.

♦ The closing overall conclusion is that with the models used in this project, the induction of moderate or mild HT in HS, with or without soft-tissue trauma, has not been found to be harmful. On the contrary, a number of potentially beneficial effects have been demonstrated.

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