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Small Volume Hypertonic Saline ±Colloid Resuscitation of Haemorrhage and Tissue Ischaemia

Experimental studies in rats and pigs

Lisbeth Waagstein

Göteborg 1998

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Akademisk avhandling

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Lisbeth Waagstein
Leg. läkare

Fakultetsopponent: **Uwe Kreimeier**, MD, PhD, München, Germany

Avhandlingen baseras på följande delarbeten

I **Waagstein LM, Wennberg E, Haljamäe H**
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Thesis to be defended January 23, 1998

Abstract

Background: Early and adequate resuscitation is of importance for the outcome of trauma victims. The traumatized tissue is a source of bleeding from damaged vessels and leakage of plasma, resulting in reduction of intravascular blood volume. Cascade systems are activated leading to increased capillary permeability and tissue perfusion. Small volume resuscitation with 4 ml/kg of body weight of hypertonic saline ± colloid has been shown to reverse severe haemorrhagic shock by improving both macro- and microcirculation. The aim of the present study was to evaluate the metabolic and circulatory effects of small volume hypertonic saline ± colloid resuscitation of haemorrhage in primary hypertension (paper I) and tissue ischaemia (paper II, III), and in paper IV even the extension of the myocardial damage.

Methods: Anaesthetized spontaneously hypertensive rats-SHR (I), Wistar rats (II), and pigs (III, IV) were studied. **I**: SHR were bled 30% of the blood volume and treated with either hypertonic glucose 42.3% (HG), hypertonic saline 7.5% (HS), or normal saline 0.9% (NS). **II**: Anterogradely perfused isolated rat hearts were paced to a heart rate of 325 beats per min and preload kept at 7.5 mm Hg while the afterload was altered from 80 to 25 mm Hg to induce ischaemia for 27 min. The hearts were exposed to either ischaemia alone, or ischaemia followed by repeated HS treatment, or repeated HS without preceding ischaemia. **III**: The efficacy of hypertonic fluid therapy on central haemodynamics, leg blood flow, and skeletal muscle metabolism at reperfusion after subtotal bilateral limb ischaemia for 240 mins was studied. Haemodynamic and metabolic alterations were followed for 180 min after reflow. **IV**: A midsternal thoracotomy was performed, and the left anterior descending coronary artery (LAD) occluded for 45 min. After a reperfusion period of 240 min biopsies from the ischaemic area were taken, the hearts excised, subjected to a staining procedure, and the left ventricle was sliced for assessment of the size of infarcted area and area at risk. In paper III and IV 4 ml/kg body weight of either NS, HS, or HS+6% dextran 70 (HSD) were administered and the infusion was started 5 min prior to reperfusion and continued during a 10 min period.

Results: **I**: All fluid regimens increased mean arterial pressure and prolonged posthaemorrhagic survival, NS more than HG (mean survival: NS 363 min; HS 170 min; HG 146 min; nontreated controls 60 min). **II**: HS administration during cardiac ischaemia induced a transient myocardial depression as also seen in nonischaemic hearts following HS. **III**: The haemodynamic support prior to and following reflow after limb ischaemia was more efficient for HS and HSD than for NS. Lactate clearance and restitution of high energy phosphagen levels in skeletal muscle were faster and more pronounced for HS and HSD. **IV**: HS depressed cardiac performance at reperfusion while HSD improved hemodynamics and myocardial contractility. HS or HSD failed to reduce the myocardial damage.

Conclusions: **I**: Small-volume HS seems superior to HG. An equal load of sodium given as NS is more effective for resuscitation after haemorrhage than HS in SHR. The sodium load, therefore, seemed more beneficial than hyperomolarity per se. **II**: Systemic rather than direct myocardial effects may be responsible for previously reported beneficial haemodynamic effects of HS in shock treatment. **III**: Small-volume HS, especially in combination with 6% dextran 70, will effectively reverse limb ischaemia-induced haemodynamic and tissue metabolic disturbances. **IV**: Administration of HSD but not HS improves haemodynamics and myocardial performance at reperfusion after 45 min of myocardial ischaemia. Neither HS or HSD reduced or increased the myocardial damage.

Key words: Haemorrhage, small-volume resuscitation, hypertonic glucose, hypertonic saline, sodium chloride, osmolality, dextran 70, spontaneous hypertension, mortality, cardiac function, coronary flow, myocardial ischaemia, myocardial metabolism, acute limb ischaemia, haemodynamics, thoracic electrical bioimpedance, skin blood flow, skeletal muscle metabolism, myocardial contractility, reperfusion, swine, area at risk, myocardial infarction.

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Dedicated

"The Salt of Life"

To Finn, Gunilla, Lars, Peter and Niklas,
and to my mother

Papers

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

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Abbreviations

AAR	Area at risk
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
$avDO_2$	Arterial-venous oxygen content difference
BE	Base excess
BSA	Body surface area
b.w.	Body weight
CF	Coronary flow
CI	Cardiac index
CO	Cardiac output
CrP	Creatine phosphate
CVP	Central venous pressure
DO_2	Oxygen delivery
dP/dt	Rate of increase in left ventricular pressure
dP/dt_{MAX}	Maximum rate of increase in left ventricular pressure
ECG	Electrocardiogram
ECF	Extracellular fluid
EDTA	Ethylenediamine tetraacetate
EVF	Erythrocyte volume fraction; haematocrit
HG	Hypertonic glucose
HR	Heart rate
HS	Hypertonic saline
HSD	Hypertonic saline 7.5% + 6% Dextran 70
IBP	Iliac artery blood pressure
LA Efflux	Cardiac lactate efflux
LAD	Left anterior descending coronary artery
LV	Left ventricle
LVP	Left ventricular pressure
LVEDP	Left Ventricular end diastolic pressure
LVSWI	Left ventricular stroke work index

MAoP	Mean aortic pressure
MAP	Mean arterial pressure
MPAP	Mean pulmonary artery pressure
MVO2	Myocardial oxygen consumption
NS	Normal saline 0.9%
O_2-Extr.	Oxygen extraction
P_aO_2	Arterial oxygen tension
P_aCO_2	Arterial carbon dioxide tension
PEEP	Positive end expiratory pressure
PCV	Packed cell volume; haematocrit
PCWP	Pulmonary capillary wedge pressure
PLSD	(Fisher´s) protected least significant difference
PVR	Pulmonary vascular resistance
RPP	Rate pressure product
SaO_2	Arterial oxygen saturation
S-ASAT	Serum-aminotransferase
S-CK	Serum-creatine kinase
S-CK-MB	Serum-creatine isoenzyme MB
S-TNT	Serum-Troponin-T
SEM	Standard error of the mean
SHR	Spontaneously hypertensive rat
SV	Stroke volume
SVI	Stroke volume index
SvO_2	Mixed venous oxygen saturation
SVR	Systemic vascular resistance
TEB	Thoracic electrical bioimpedance
TFI	Thoracic fluid index
TTC	Triphenyltetrazolium chloride
VO_2	Oxygen consumption

Contents

1. Introduction

Fluid Resuscitation of Shock and Tissue Ischaemia

In the past years it has become obvious that a key factor rendering patients to develop multiple systems organ failure (MSOF) after shock and ischaemia is the persistence of impaired microcirculation with tissue hypoxia and deterioration of cellular function.[1] Prehospital, perioperative and intensive care related efforts aim at the reduction of trauma deaths by efficient resuscitation from hypovolaemia and rapid reversal of the systemic hypotension and restoration of *oxygen delivery* to the tissues.[1] Although the initial treatment of trauma patients is focused on measures to eliminate life-threatening airway blocking, support of ventilation and oxygen delivery (DO_2), fluid therapy is an important component of the resuscitation. The optimal composition and amount of fluid for resuscitation has been discussed in decades and factors such as haemostasis, tissue oxygenation, cell metabolism, haemodynamics, and microvascular blood flow have been considered in detail. Quite often a crystalloid-colloid controversy in the treatment of trauma patients has been focused on.[2] However, more and more interest has during the last decade been focused on the potential value of small-volume (4 ml/kg body weight) resuscitation with hypertonic (7.5%) sodium chloride (2.400 mOsm/kg).[3,4]

Definition of Small Volume HS Resuscitation

Hypertonic Resuscitation is by Kramer in the Hypertonic Resuscitation Website defined as - "the infusion of hypertonic or hyperosmotic fluid to improve cardiovascular function in an animal or man suffering from shock or circulatory dysfunction".[5]

Small Volume Resuscitation is usually given as a bolus infusion of about 10% of the estimated blood volume deficit or 4 ml/kg b.w. of hypertonic (7.5%) or hypertonic-hyperoncotic saline solution within 2-5 min through a peripheral vein for primary resuscitation from severe hypovolaemia generally seen after trauma and haemorrhage.[1,6]

Nakayama et al[6] introduced the term "small-volume resuscitation" in 1984 when they were able to show that a bolus injection given over 60 s of HS 7.5% equal to 10% of shed blood volume rapidly normalized blood pressure and cardiac output in haemorrhaged conscious sheep. These improvements were however transient.

Historical Background

Hypertonic solutions have been used since the first part of this century (Penfield[7] 1919; Read et al.[8]1961; Baue et al.[9] 1967; Wildenthal et al.[10] 1969; McNamara et al.[11,12] 1970, 1972; Wolf[13] 1971) and among the described effects positive inotropy and vasodilation were considered beneficial. Possible disadvantages due to a hypotensive effect, accentuated by increasing the rate of injection as well as by raising the concentration (5% NaCl, 12,5% urea in saline) was reported by Read et al[8]. It was also noted in several of the above reports that the beneficial effects were transient in the treatment of haemorrhagic hypotension.

In 1980 Velasco et al[14] reported on the administration of highly hypertonic saline (HS 7.5%, 2400 mOsm/l) to dogs subjected to haemorrhagic shock. The infused volume was very small (4 ml/kg body weight), and equal to 10% of the shed blood volume. HS rapidly restored arterial pressure and cardiac output to base-line values and resulted in 100% survival. The control group resuscitated with normal saline (0.9%) had no survivors. This study was followed by several others and the concept of "small volume resuscitation" was constituted.[6] In addition to resuscitation of haemorrhagic shock there are several studies which show the efficacy of HS in the treatment of burn injury, head trauma, endotoxin shock, and in ischaemia-reperfusion situations as summarized in recent reviews by Rocha e Silva[3] and Kramer et al.[4]

The first study investigating the use of HS in combination with a colloid (addition of 6% dextran 70) was described by Smith et al[15] (1985) and it was followed within the next 5 year period by a number of experimental and clinical studies Maningas[16,17] (1986,1989), Kreimeier et al[18] (1987), Holcroft et al[19] (1987), Velasco et al[20,21] (1987, 1989), Kramer et al[22] (1989), Wade et al[23,24] (1989, 1990). In the resuscitation of haemorrhaged animals, these studies confirmed that the combination of HS and dextran (HSD) was superior to HS alone. HSD induced a higher cardiac output, slightly better and more sustained plasma volume expansion, and increased survival rates.

The first clinical study on HS in the treatment of shock appeared in 1980 and was carried out by DeFelippe et al.[25] In the study 12 patients in refractory hypovolaemic shock were treated with small volumes of HS (maximum 200 ml) and a significant pressor response with recovery of consciousness and urine output was seen in 11 of the patients. Since then the clinical efficacy of HS and HSD in the treatment of shock and trauma patients has been assessed in detail in a number of randomized double blind studies. Holcroft et al[19] (1987), and Younes et al[26] (1992) used prehospital infusion of 250 ml of HSD in trauma patients and reported that the treatment improved the clinical outcome. Since then a number of studies have been performed as recently summarized by Kramer et al[4] (1997). In a meta-analysis of all available studies Wade et al[27] (1997) found that treatment with HSD resulted in a 3.6% higher discharge survival rate than treatment with standard fluid regimens.

Physiological Mechanisms of HS

The different main effects of HS treatment are summarized in Table 1 (Haljamäe et al, 1995).[28]

Table 1. Effects of HS treatment.

Fluid redistribution
• increased intravascular volume
-haemodilution
-reduced blood viscosity
-increased venous return
-increased preload
-increased cardiac output
Vasodilation
• reduced afterload
-improved regional blood flow
-reduced cardiac work
Cellular deswelling
-improved capillary blood flow
-reduced tissue oedema
Direct cellular membrane effects
• central sympathetic activation
• cellular functional alterations

HS induces a rapid mobilization of fluid from the extra- and intracellular compartments into the intravascular compartment resulting in a dynamic support of central haemodynamics.[29] This fluid redistribution, caused by the osmotic HS gradient, is similar to the physiological transcapillary fluid mobilization that is induced by the hyperglycaemic response to shock and trauma.[30] The ability to increase blood glucose and maintain hyperglycaemia has been related to increased survival after haemorrhage. However, the circulatory effect induced by 7.5% HS is more pronounced. In spite of the fact that an initial physiological glucose-osmotic transcapillary refill has already taken place as part of the early physiological response to shock- or trauma there will be an additional pronounced increase in plasma volume in response to infusion of HS solutions.[29]

An important functional role of concentrated sodium ions has been demonstrated[31,32] and thereby the relative importance of osmolality as compared to ionic composition of hypertonic solutions in shock treatment. Electrolyte solutions based on cations other than sodium or anions other than chloride do not reverse haemorrhagic shock and improve survival rates as efficiently as hypertonic sodium chloride.[31] Hypertonic glucose has e.g. a less pronounced intravascular volume expanding and arterial blood pressure supporting effect than HS. A greater volume expanding effect from HS as compared to hypertonic glucose may be explained by a distribution of sodium ions mainly within the extracellular space, while glucose is also taken up and metabolized by tissue cells. Hypertonic glucose seems in addition to cause an increased lactic acid load due to enhancement of anaerobic glucose metabolism.[32] The importance of the sodium ion as compared to osmolality *per se* for the efficacy of hypertonic fluid therapy in shock is consequently well documented and a high plasma sodium level seems essential for survival.[28,32]

The HS induced haemodilution caused by the dynamic fluid redistribution offers haemorheological advantages seen as improved blood flow through the terminal vascular bed and an enhanced venous return.[33] There is an efficient restitution of organ perfusion following HS infusion, especially if a hypertonic-hyperoncotic fluid combination rather than HS alone is chosen.[34] A deswelling of blood cells and vascular endothelial cells will occur following infusion of HS in addition to the direct vasodilatory effects of HS.[35]

HS solutions in the treatment of hypovolaemic conditions improve cardiac output.[6,14,19,31,36-39] Although results from studies of intact animals suggest that myocardial contractility may be increased by HS[6,14,40,41] there is still only limited information available on the direct myocardial effects of HS on shock- and ischaemia-induced cardiac dysfunction. A number of the available studies indicate that the direct effects of HS on myocardial performance may be depressant rather than stimulatory.[8,42-45] There are data suggesting that the contractility is decreased as well as blood flow in the ischaemic myocardium by HS infusion, while in the normal myocardium both coronary blood flow and contractility increase.[46] This could lead to worsening of ischaemic injury, which may be deleterious in hearts with impaired contractile function caused by ischaemia.[46] However, the extension of myocardial damage following HS or HSD administration after acute myocardial infarction has so far not been reported.

Effects of the Dextran Component

Since the volume supporting effect of HS is transient, the use of colloids in addition to HS has been evaluated. It was early established that the usual combination of 7.5% hypertonic saline-6% dextran 70 (HSD) of 4ml/kg body weight in the resuscitation of haemorrhagic hypotension was efficient. In the first 10-30 minutes after infusion HS alone[47] and HSD expands plasma volume 3-4 times the infused volume, but only HSD sustains the plasma volume expansion after 3 hours.[4] This is to compare with isotonic 0.9% saline which is a poor volume expander as only one-third of the infused volume remains in circulation after 2 hours.[4] The plasma volume expansion effect induced by HSD results in improved haemodynamic function and survival in otherwise lethal animal models of haemorrhage.[16,21,23]

Nolte et al demonstrate that HSD (bolus infusion of 7.2 % NaCl/10% dextran 60) effectively attenuates postischaemic microvascular disturbances in striated muscle elicited by ischaemia-reperfusion, presumably through reduction of postischaemic leucocyte-endothelium interaction (i.e. reduces leucocyte rolling and sticking in the microcirculation). HSD also effectively attenuates macromolecular leakage and reduces capillary endothelial swelling as assessed by measurements of capillary diameters. HS alone was significantly less efficient in protecting from post-ischaemic leucocyte-endothelial interaction.[48]

2. Aims of the Present Study

The aims of the present study were experimentally to evaluate the circulatory and metabolic effects of:

I Small volume hypertonic saline as compared to non-ionic hypertonic solution (glucose) in the resuscitation of haemorrhage in animals with reduced shock tolerance (spontaneously hypertensive rats - SHR) *(paper I)*.

II Hypertonic saline on the non-ischaemic and ischaemic myocardium by the use of an isolated working rat heart model *(paper II)*.

III Small volume hypertonic saline ± colloid as compared to isotonic saline treatment of acute limb ischaemia (simulated aortic saddle embolism in pigs) *(paper III)*.

IV Small volume hypertonic saline ± dextran treatment of acute regional myocardial ischaemia (LAD occlusion in pigs) *(paper IV)*.

3. Materials and Methods

Experimental Animals

All experiments were approved by the Animal Ethics Committee of the University of Göteborg and conducted in accordance with the National Institute of Health guidelines for the use of experimental animals.

The investigations were performed in:

Paper I: Thirtytwo 5-6 months old male Spontaneously Hypertensive rats (SHR) weighing 330-430 g.

Paper II: Thirtytwo 3-4 months old Wistar rats of either sex, weighing 300-430 g.

Paper III: Twentyfour crossbreeding pigs (Swedish landrace, Yorkshire, and Pigham) of either sex, weighing 26-50 kg.

Paper IV: Thirtyfour crossbreeding male, not castrated pigs (Swedish landrace, Yorkshire, and Hampshire) of three months of age, weighing 18-28 kg.

The rats had free access to food and water until the start of the experiments *(I, II)*. The pigs were fasted for 15 h prior to the experimental procedures but had free access to water until the experimental day *(III, IV)*.

All pigs were premedicated with pethidine hydrochloride (Petidin®) 50 mg *(III)* and 40 mg plus 1 mg of atropine (Atropin®) *(IV)* intramuscularly 30 min before transfer to the operating room.

Anaesthesia and Surgical Procedures

Four different haemorrhagic shock or ischaemia associated experimental conditions were studied.

Paper I:

Anaesthesia was achieved by an intraperitoneal dose of the short-acting barbiturate, pentobarbital sodium (60 mg/kg body weight), and if needed additional doses (9-12 mg/kg) were given during the experiment. A polyethylene catheter (PE 50) was inserted into one of the femoral arteries for bleeding, and blood sampling, as well as recording of mean arterial pressure and heart rate. The catheter was flushed with 250 units of heparin to prevent clotting. One jugular vein was cannulated for intravenous infusions. The animals were placed in the lateral position to keep free airways and breathed room air.

Paper II:

The animals were anaesthetised with a single intraperitoneally administered dose of an ultra-short-acting barbiturate, methohexital (Brietal®) 75-100 mg/kg b.w. Heparin 1000 IU/kg b.w. was at the same time administered in order to reduce the risk of intravascular thrombotic events due to activation of the coagulation system during the preparative surgical procedure for the isolation of the heart. A thoracotomy was rapidly performed and the heart was excised and put into ice-cold saline, which stopped the functional activity within a few seconds. The aorta, which was transected about 4-5 mm above the aortic valves, was immediately mounted on a steel cannula and retrograde perfusion of the coronary arteries started usually within 30 sec from the time of excision. During the retrograde perfusion the left atrium was connected via one of the pulmonary veins to an angled steel cannula, while the other pulmonary veins were ligated *(Figure 1)*. Antegrade perfusion was started by clamping the tube from the pre-perfusion reservoir and unclamping the tube connected to the left atrium. The preparative procedure for antegrade perfusion has previously been described in detail.[49]

The perfusate was an oxygenated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate buffer containing (mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, di-NaEDTA 0.5, and glucose 14. The temperature of the perfusate was kept at 37° C.

The antegrade perfusion apparatus consisted of a 130 cm long oxygenation chamber from which the perfusate passed via a micropore filter and duplicate bubble traps to the left atrium. The preload of the left atrium could be adjusted by changing the height of the bubble trap immediately proximal to the heart and the pressure level was measured and recorded via a side-tube connected to a Statham 23 DC transducer and a Grass polygraph recorder (model 7 D, Grass Instrument Co. Quincy, MA, USA). The aortic pressure against which the heart pumped was set at the desired level by means of a Starling resistor[49,50] and the pressure was measured and recorded just above the aortic valves via a thin steel cannula and PE-10 tubing (Statham 23 DC transducer, Grass polygraph recorder). A windkessel effect on the aortic outflow was achieved by attaching the aortic cannula to a 8 mm wide and 40 mm long Penrose drain rubber tube interconnected to a 2 ml syringe containing 1 ml of air and 1 ml of saline *(Figure 1)*.

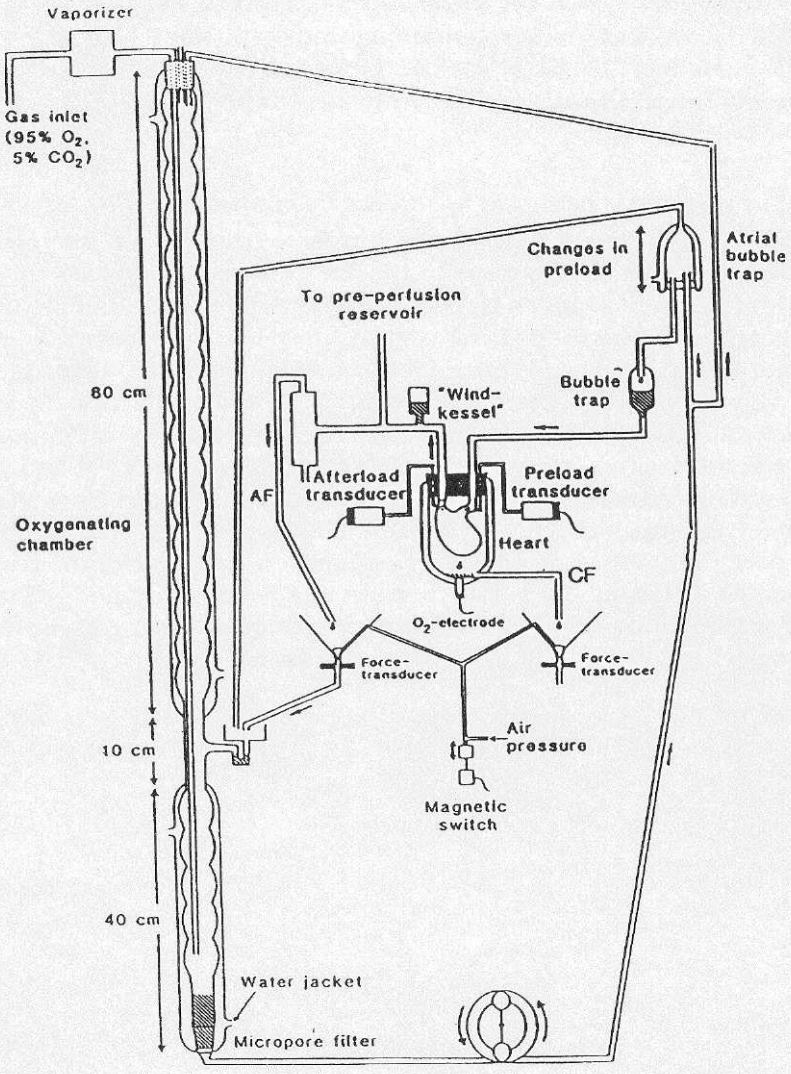

Figure 1. *Schematic illustration of the experimental setup for antegradely perfused hearts. Left atrial pressure and aortic pressure could be altered independently. The hearts were electrically paced. Aortic flow (AF) and coronary flow (CF) were collected and intermittently weighed in separate funnels by means of a force transducer and balloons in the funnels driven by a magnetic switch and an air pressure system. Oxygen tension was continuously measured in the coronary venous effluent. (Figure redrawn from Sahlman L et al[49] with permission from Anesthesiology).*

Paper III:

Anaesthesia was induced with ketamine hydrochloride (Ketalar®) 1000 mg given intramuscularly. An ear vein was then cannulated and methomidate (Hypnodil®) 100 mg and azaperone (Stresnil®) 40 mg were administered intravenously. Anaesthesia was maintained with supplementary doses of methomidate and azaperone when needed and a basal fluid infusion of normal saline (0.9 % NaCl) was administered at a rate of 30 ml/kg/24 h. A prophylactic dose of the antibiotic, Cloxacillinsodium (Ekvacillin®, Astra, Södertälje, Sweden) 1000 mg was administered intravenously prior to the surgical procedure to prevent wound infection. The animals were placed on their right side and breathing spontaneously. In this position a vascular graft was inserted as previously described.[51] A left lumbar incision was made followed by minor retroperitoneal dissection for visualisation of the lateral aortic wall. A partially occluding vascular clamp was applied on the visible lateral aspect of the aorta approximately 5 cm proximal to the aortic bifurcation. An 8 mm preclotted dacron vascular graft was then sutured end-to-side to the aorta, using 4/0 Prolene. Care was taken to avoid damage to periarterial vessels, nerves, and lymphatics during this procedure. The outer end of the vascular graft was closed with a ligature and placed subcutaneously. The wound was closed and infiltrated with bupivacaine hydrochloride 5 mg/ml, adrenaline 5μg/ml (Marcain® adrenalin) 10 ml for postoperative pain relief and the animals were allowed to recover.

Approximately one week later the acute experiment took place. The pre-experimental handling of the animals was identical to that at the insertion of the vascular graft. Anaesthesia was maintained and the basic needs for fluid requirement satisfied by i.v. infusion of 500 ml 0.9% NaCl containing 3000 mg of methomidate, 800 mg of azaperone, and 100 mg of pethidine. The infusion rate was set at 30 ml/kg/24 h. Supplementary doses of methomidate and azaperone were given when needed during the experiment. After tracheostomy via a midline neck incision, the animals were mechanically ventilated with 30% oxygen in room air using a Servo ventilator 900 (Elema Schönander, Sweden). The ventilated minute volume was set to keep P_aCO_2 at 4.5-5.5 kPa. An indwelling catheter was inserted suprapubically into the urinary bladder. A catheter was placed into the superior caval vein via the left external jugular vein for measurements of the central venous pressure and for fluid infusions.

A triple-channel balloon catheter (Pruitt venous thrombectomy catheter 7 F. Ideas for Medicine, INC. Tampa, FL, US) with one lumen proximal and the other distal to the balloon was introduced into the distal abdominal aorta via the previously inserted graft.

The balloon was placed immediately above the aortic bifurcation and inflated in order to induce acute occlusion of the distal aorta *(Figure 2)*.

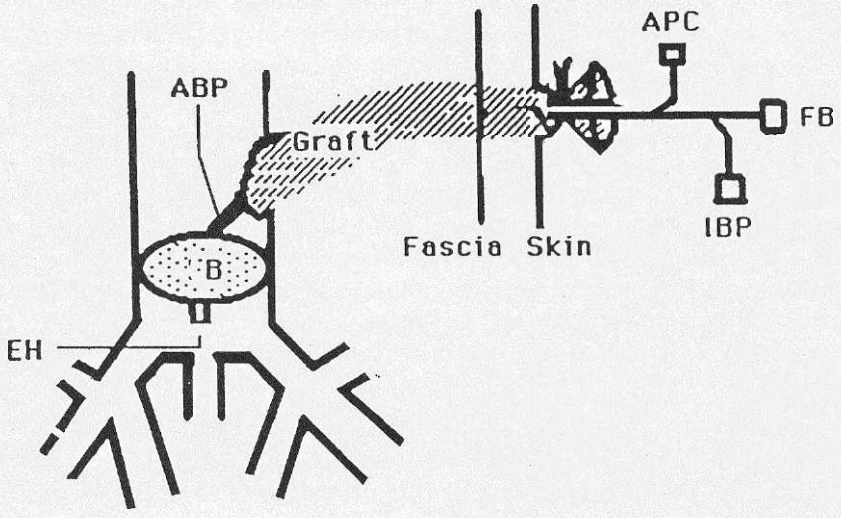

Figure 2. *Schematic illustration of the experimental model for acute distal aortic occlusion. ABP = Aortic blood pressure channel, inner end. APC = Aortic blood pressure channel, outer end. B = balloon. FB = Filling channel of the balloon catheter. EH = end hole of the balloon catheter. IBP = channel for iliac blood pressure. (Figure modified from Jivegård L et al[51] with permission from European Journal of Surgery).*

Paper IV:

The induction and maintenance of anaesthesia as well as the basal fluid administration were similar as in paper III with the exception of lower induction doses of methomidate (50 mg), and azaperone (12 mg). The procedures for tracheostomy and ventilation were also similar as in paper III.

A venous line was established via a vein in the right hind limb to administer the treatment fluids. An arterial line was placed into the left femoral artery for blood sampling and blood pressure monitoring and a fiberoptic five-lumen pulmonary artery catheter (OPTICAT®, 5.5 F. Abbot Laboratories North Chicago, U.S.) was placed into the pulmonary artery via the right external jugular vein and used with the Oximetrix® 3 System SO_2/CO Computor, Abbot Laboratories North Chicago, U.S.) that displays venous oxygen saturation and thermodilution cardiac output curves, and calculates, from standard formulae, several hemodynamic variables. The pressures were recorded via pressure transducers (Dispo-sense® Peter von Berg GmbH, München, Germany) and a monitor (Model 78342, Hewlett Packard, MA, U.S.). A high fidelity pressure measuring catheter 8 F consisting of two sensors with one sensor in the left ventricle and the other one in the aorta, was placed into the right carotid artery and retrogradely inserted into the left ventricle (LV) for the measurement of LV systolic pressure, LV end-diastolic pressure, +dP/dt and aortic pressure. The catheter was connected to two pressure interfaces (SENTRON® Pressure Measurement System. Cordis Corporation, Miami, FL, U.S.). ECG and all pressures were recorded by a model 7 D, Grass polygraph recorder (Grass Instrument Co. Quincy, MA, U.S.). During the experiment all catheters were continuously flushed with heparin 5000 IU in 500 ml of NaCl 0.9% to prevent clotting. Animals showing signs of disease (e.g. pneumonia or pericarditis) when chest was opened, iatrogenic complications during the surgical procedure, depressed left ventricular function, or ventricular fibrillation resistant to defibrillation during coronary occlusion or at reperfusion were excluded from further evaluation.

Surgical Procedure and Induction of Myocardial Ischaemia

The model for the surgical procedure and myocardial infarct size quantification used in this study has previously been described.[52] Ten min after the catheterizations were finished baseline data were collected. Thereafter a midsternal thoracotomy was performed and a 5 mmHg positive end-expiratory pressure (PEEP) was established.

The pericardial sac was opened and a 1 cm section of the left anterior descending artery (LAD) dissected free from the epicardium just distal to its first diagonal branch and topical lidocaine (2%) was applied to prevent vasospasm during LAD isolation. A snare was placed under the isolated LAD and this was used for a slow removal of the occlusion to prevent reperfusion arrythmias. Heparin (1000 IU) was administered intravenously to prevent clotting. A stabilization period of 30 min was allowed before pre-LAD-occlusion data were collected.

The LAD was then occluded for 45 min using a microaneurysm clip and the occlusion was confirmed by observation of regional cyanosis and ventricular systolic bulging distal to the LAD-occlusion in the corresponding area of the myocardium. Reperfusion after 45 min of occlusion was established by removing the clip and confirmed by gradual (< 2 min) disappearance of the cyanotic ischaemic zone. Prior to the occlusion and reperfusion i.v. injection of Ketalar® 25-75 mg was given to reduce stress and the risk of ventricular fibrillation.

Measurements and Recordings

Paper I:

Arterial blood pressure and heart rate were continuously recorded from one of the femoral arteries by a transducer (Gould P 23 1 D) and recorded on a recorder (Grass model 7 D Polygraph Grass Instrument Co. Quincy, MA). Rectal temperature was monitored and the rats were warmed with a heating pad when the temperature fell below 36° C.

Paper II:

Left atrial pressure (LAP), mean aortic pressure (MAP), heart rate (HR), aortic flow (AF), coronary flow (CF), and PO_2 of the coronary venous effluent were continuously recorded (Grass model 7 D Polygraph Grass Instrument Co. Quincy, MA). Aortic flow was determined by collecting the outflow in a funnel placed on top of a transducer (FT 10, Grass Instruments) and weighing the outflow intermittently. The intermittent weighing and emptying of the funnel was regulated by an automatic, time-controlled, magnetic switch (collection and weighing time 6 secs; emptying time 4 secs). The coronary flow was allowed to flow freely onto a Clarc PO_2 electrode (Radiometer, Copenhagen, Denmark) before it was collected in a separate funnel and weighed (Figure 1). PO_2 in the perfusate was measured intermittently. Cardiac output was calculated as the sum of the aortic and coronary flows. Stroke volume (SV) was calculated and expressed in μl/100 g of body weight. Myocardial oxygen extraction (%) was determined as:

$$PO_{2LA}\text{-}PO_{2V} / PO_{2LA},$$

and oxygen consumption (MVO_2) was calculated as previously described.[49]

Paper III:

Aortic pressures above and below the occlusion were measured via the proximal and distal ports (Figure 2). Heparin (5000 IU) was administered intravenously. The proximal and distal aortic, and central venous pressures were measured and recorded continuously (pressure transducers dispo-sense® Peter von Berg GmbH, München, Germany; pressure monitor model 78342, Hewlett Packard, MA, US; recorder Multitrace 4, Lectromed, UK). An electrocardigram (ECG) was continuously monitored and recorded.

A standard laser Doppler probe (Pf 108 d, Perimed, Sweden) was attached to the plantar surface of the right and left hind feet and connected to a Periflux PF1d Laser Doppler Flowmeter (Perimed KB, Stockholm, Sweden) for assessment of skin blood flow.[51,53]

Cardiodynamic data were obtained by thoracic electrical bioimpedance (TEB) measurements using a non-invasive continuous cardiac output monitor (NCCOM3-R6, Bomed Medical Manufacturing Ltd, Irvine, CA, US).[54] The following data were obtained and registered: heart rate (HR), cardiac output (CO), stroke volume (SV), and thoracic fluid index (TFI). Calculated data were cardiac index (CI), stroke volume index (SVI), and systemic vascular resistance (SVR).
Rectal temperature was measured intermittently and the animals were kept at a body temperature of about 38° C - 39° C by external heating.
The weight of the animals was registered before and after the experiment to allow calculation of changes in body weight.

Paper IV:

The following data were measured and recorded throughout the experiment: Lead II electrocardiogram (ECG); body temperature (blood: Oximetrix® 3 System SO_2/CO Computer Abbot Laboratories North Chicago, U.S) was registered intermittently and the animals were kept at about 37.5° C - 38,5° C by external heating; heart rate (HR); systolic, diastolic, and mean arterial pressure (MAP); systolic, and end-diastolic LV pressure (LVEDP); +dP/dt; systolic, diastolic, and mean aortic pressure (MAoP); central venous pressure (CVP); systolic, diastolic, and mean pulmonary artery pressure (MPAP); pulmonary capillary wedge pressure (PCWP). Haemodynamic data for the calculations were based on the mean value of triplicate measurements at the end of the expiratory phase of the respiratory cycle. Rate pressure product (RPP = HR x MAP) and left ventricular stroke work index [LVSWI = SVI (MAP-PCWP) · 0.0136] were calculated.
From the Oximetrix® 3 System SO_2/CO Computer and Printer the data were obtained for: body surface area (BSA); cardiac output (CO) using the thermodilution technique (three accepted measurements at injection of 3 ml of 5% Glucose at room temperature); cardiac index (CI); mixed venous oxygen saturation (SvO_2); systemic vascular resistance (SVR); pulmonary vascular resistance (PVR); arterial-venous oxygen content difference ($avDO_2$); arterial oxygen content (CaO_2); mixed venous oxygen content (CvO_2); oxygen delivery (DO_2); oxygen consumption (VO_2).
The following calculations were performed: Oxygen extraction (O_2 - extr.) = $avDO_2/CaO_2$, and stroke volume index (SVI) = CI/HR.
The animals were weighed before and after the end of the experiment to calculate changes in body weight.

Laboratory Analyses

Paper I:

For blood glucose and lactate determination perchloric acid (3M) was added to the blood samples (0.05 ml). After neutralization with $KHCO_3$ (2M), the extracts were centrifuged (3000·g) and the supernatants analysed for glucose and lactate using an enzymic fluorometric assay.
For determination of serum osmoles 0.3 ml of blood was sampled in a heparinized tube and analysed using a vapor osmometer (WESCOR 5500 vapor pressure osmometer, Teknisk, Kjemisk a/b Wa Mo BERGEN, TØNSBERG, Norway).[55] For determination of haematocrit, serum sodium, blood gas and acid-base state 0.25 ml was sampled in 1 ml syringes prefilled with heparin lithium (CONCORD LABORATORIES LTD, Kent, England) and analysed with B G Electrolytes Analyzer (Il BGE, Instrumentation Laboratory Spa, Milano, Italy).

Paper II:

The coronary venous outflow was collected during 3 min periods (A-E; cf Figure 3) and the lactate concentration determined fluorometrically as previously described[56] and the myocardial lactate efflux was calculated . The electrolyte content of the perfusate and the coronary venous outflow was analysed using a Radiometer KNA2 analyzer (Copenhagen, Denmark). Osmolality of the coronary venous outflow was determined using a Cryomatic Osmometer Model 302 (Advanced Instruments Inc., Needham Heights, MA).

Paper III:

Arterial blood was sampled from the proximal channel of the aortic balloon catheter for analyses of haemoglobin (photometric method), haematocrit (microhaematocrit), and serum osmolality (freezing point depression, osmometer - Advanced Instruments Inc. Needham Heights, MA, US). Serum protein, sodium, potassium, and chlorides were analyzed with Kodak Ektachem Analyzer DT 60 and DTE-Modul (Eastman Kodak Company, NY, US) and blood gases with a blood gas analyzer (ABL 500, Radiometer, Copenhagen, Denmark).

Paper IV:

Arterial blood was sampled from the femoral artery catheter for blood chemistry analyses (B G Electrolytes Analyzer (II BGE, Instrumentation Laboratory Spa, Milano, Italy); haematocrit (Hct) (conductivity); haemoglobin (Hb) (estimated from measured haematocrit); sodium (Na^{+}); potassium (K^{+}); ionized calcium (Ca^{++}); hydrogen ion activity (pH); carbon dioxide partial pressure (pCO_2); oxygen partial pressure (pO_2); and calculated parameters: base excess (BE); standard bicarbonate (SBC); and oxygen saturation (sO_2). Corrected at the animals body temperature are pH, pCO_2 and pO_2. Serum chlorides (Cl^{-}), blood glucose, and total protein were analysed with Kodak Ektachem Analyzer DT 60 and DTE-Modul (Eastman Kodak Company, NY, US), and serum osmolality (freezing point depression, osmometer - Advanced Instruments Inc. Needham Heights, MA, US). Blood lactate was analysed using an enzyme fluorometric assay.

Serum Markers of Myocardial Necrosis

Prior to, during, and after the induced myocardial ischaemia the following serum markers for myocardial necrosis were analyzed: serum-aspartate aminotransferase (S-ASAT) and serum-creatine kinase (S-CK) determined by a spectrophotometrical assay; serum-creatine kinase isoenzyme MB (S-CK-MB) determined by immunological methodology (IMx CK-MB, Abbot Laboratories Diagnostic Division, Abbot Park, North Chicago, Illonois, USA); serum-Troponin-T (S-TNT) by immunometrical methodology (Enzymun-Test®, Boehringer-Mannheim, Germany). The S-CKMB/CK ratio was calculated.

Tissue Analyses

Paper III:

Skeletal Muscle Metabolites and Water Content

In this study skeletal muscle biopsies were rapidly excised from the distal part of the gastrocnemic muscle and and immediately frozen in liquid nitrogen. The biopsies were homogenized in icecold perchloric acid (3 M), centrifuged (2000 g), and the extracts were neutralized with $KHCO_3$ (2 M). Thereafter a second centrifugation was performed, separating the supernatant from the precipitate. The supernatants were used for fluorometric analyses of adenosine triphosphate (ATP), creatine phosphate (CrP), and lactate, and the pellets for determination of the protein content using previously described techniques.[56] The metabolite levels were expressed as μmol/g protein.

A second muscle biopsy was taken for determination of the water content by weighing prior to and after heat drying (desiccator at 70° C for 24 hours). The water content was calculated and expressed as a percentage.

Paper IV:

Myocardial Muscle Metabolites

Cardiac muscle biopsies were rapidly excised from the damaged myocardial area at the end of the registered reperfusion period (R 240 min) and immediately frozen in liquid nitrogen. The methods used for the analyses of the biopsies were similar as in paper III. The supernatants were used for fluorometric analyses of adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), creatine phosphate (CrP), lactate, and glucose, and the pellets for determination of the protein content.[56] The metabolite levels were expressed as μmol/g protein.

Myocardial Infarct Size Quantification

At the end of the experiment the heart was rapidly excised and immersed in 37° C warm saline. Myocardial infarct size was quantified using Evan´s blue (E. Merck, Darmstadt, Germany) and triphenyltetrazolium chloride (TTC, E. Merck, Darmstadt, Germany). Two catheters, connected to syringes containing Evan´s blue (120 ml; 0.4%), were inserted into the ostia of both coronary arteries, while the tip of another catheter, connected to a syringe containing TTC (60 ml; 1% dissolved in 0.01 M Phosphate buffer at 37° C), was inserted into the LAD at the site of occlusion. The injection of TTC was started before and ending after the injection of Evan´s blue. All fluids were injected rapidly from the syringes into the heart. The heart was placed in 37° C saline for 15 min, then 0.8-1.0 cm thick left ventricular slices were obtained.

By this staining procedure, normal myocardium stained blue, area at risk stained red, while infarcted myocardium remained unstained. The slices were photographed (Hasselblad polaroid camera 503 CX & 500 C/M, Victor Hasselblad AB, Göteborg, Sweden) *(Figure 3)* and the normal, at risk and infarcted areas were quantified by two independent individuals using a planimetric method (NIH Image 1.54 processing and analysis program for the Macintosh) and an area measurement protocol. The mean value of the areas measured by the two individuals were used. The coefficients of correlation between the measurements made by the two independent individuals were 0.91 for Area at Risk (AAR) % of left ventricle, and 0.97 for infarct size % of AAR, and 0.94 for infarct size % of left ventricle.

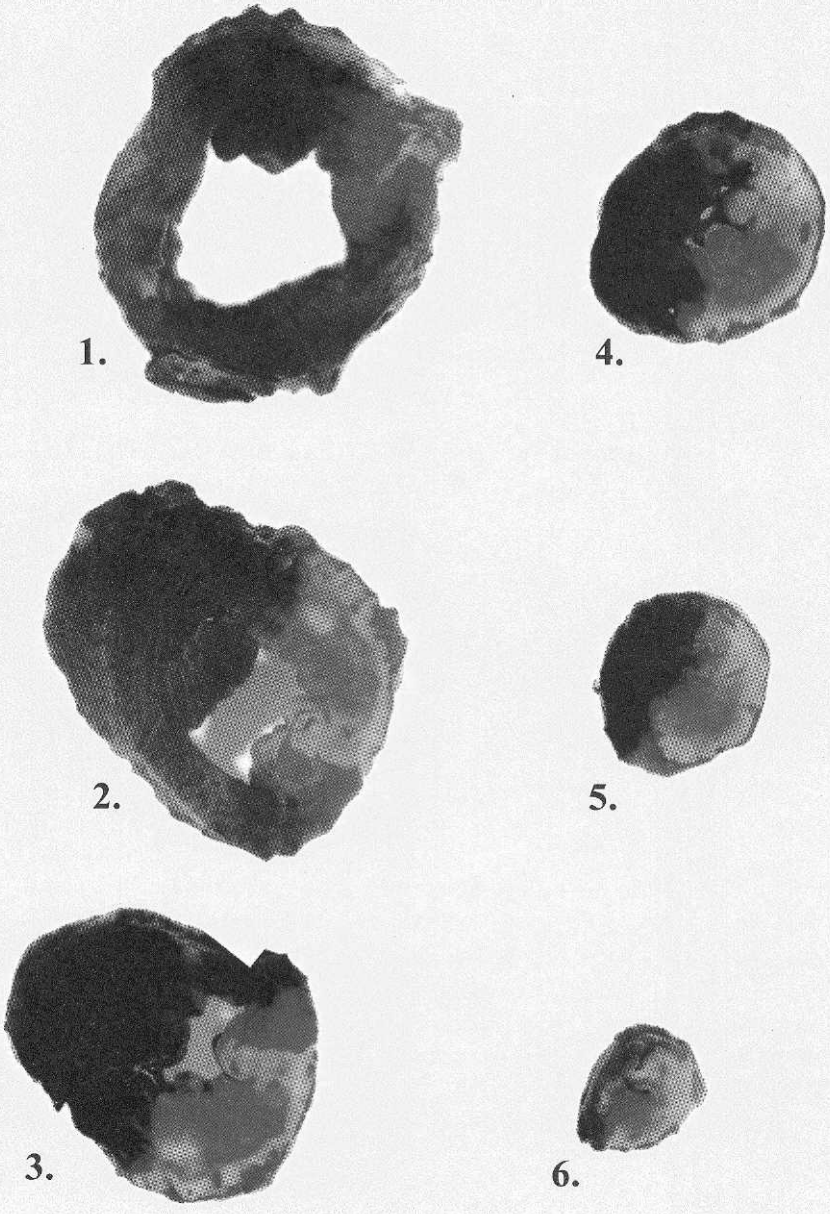

Figure 3. TTC- and Evan´s blue-stained slices of the left ventricle in one pig. Blue area denotes normal myocardium, while viable myocardium (Area at Risk) is delineated with its bright red colour from the unstained (yellow-white) infarcted myocardium.
1-6 show from the base to apex.

Experimental Procedures and Treatment

Paper I:

Acute blood loss of the SHR was induced after a steady state period of at least 10 min after the preparative procedures. 30% of the calculated blood volume (6% of body weight) was withdrawn during a 20 min period. After 5 min from the end of bleeding the animals were treated during a 10 min period according to three different fluid regimens, and a fourth group served as control (n=8 in each group).

1. ***Hypertonic saline*** (HS 7.5%; 2,400 mOsm/l), 25% of shed blood volume = 4.5 ml/kg b.w.

2. ***Hypertonic glucose*** (HG, 42.3%; 2,400 mOsm/l), 25% of shed blood volume = 4.5 ml/kg b.w.

3. ***Normal (0.9%) saline*** (NS), to provide equal sodium load as in HS = 37.5 ml/kg b.w. (volume = 2.1 times shed blood volume).

4. ***No treatment*** to a control group (C).

Blood was sampled at steady state, after bleeding, and 30, 90, 210, 330, 450, 510, 570, 630, and 690 min after treatment for determination of packed cell volume (PCV), blood glucose, blood lactate, serum sodium, serum osmoles, blood gas and acid-base state. At each sampling 0.8 ml of blood was drawn and the withdrawn volume was compensated for by infusion of an equal volume of isotonic saline. Sampling at steady state and immediately after bleeding was included in the shed blood volume. Spontaneous survival time after bleeding and treatment was followed.

Paper II:

The function of isolated, atrial paced rat hearts (325 beats/min by a square-wave stimulator at a pulse duration of 4 msecs and 3 volts), which were perfused by an antegrade technique[49] at a filling pressure (left atrial pressure) of 7.5 mm Hg, was assessed in three different experimental groups. A 10 min stabilization was always allowed followed by a 3-min control period (*Figure 4*, period A).

Ischaemia plus Hypertonic Saline Group (n = 12):

Basal haemodynamic and metabolic data were collected during the initial 3-min control period (*Figure 4*, period A), at a mean aortic pressure of 80 mm Hg. Thereafter, myocardial ischaemia was induced by reducing mean aortic pressure during a 1-min period to 25 mm Hg and maintaining this low mean aortic pressure value for 27 mins. After an initial 6-min period of ischaemia a 3-min data collection period (*Figure 4*, period B) took place. Thereafter, heated (37° C) hypertonic saline (3%) was added to the perfusate to increase the sodium concentration to ~150 mmol/l. After 6 mins had passed, there was a third 3-min data collection period (*Figure 4*, period C) followed by a second addition of hypertonic saline to reach a sodium concentration of the perfusate of ~160 mmol/l. Myocardial ischaemia was maintained for another 9-min period, including a 3-min period of data collection (*Figure 4*, period D). After the period of myocardial ischaemia mean aortic pressure was normalized during a 1-min period to 80 mm Hg. This mean aortic pressure value was maintained during an additional 9-min period, including a data collection period (*Figure 4*, period E).

Control - Ischaemia Group (n = 8):

Ischaemia was induced as in the ischaemia plus HS group but no HS was administered.

Control - Hypertonic Saline Group (n = 12):

In this group, there was no reduction of aortic pressure. However, HS was added to reach a sodium concentration that was similar to the sodium concentration of the ischaemia plus HS group.

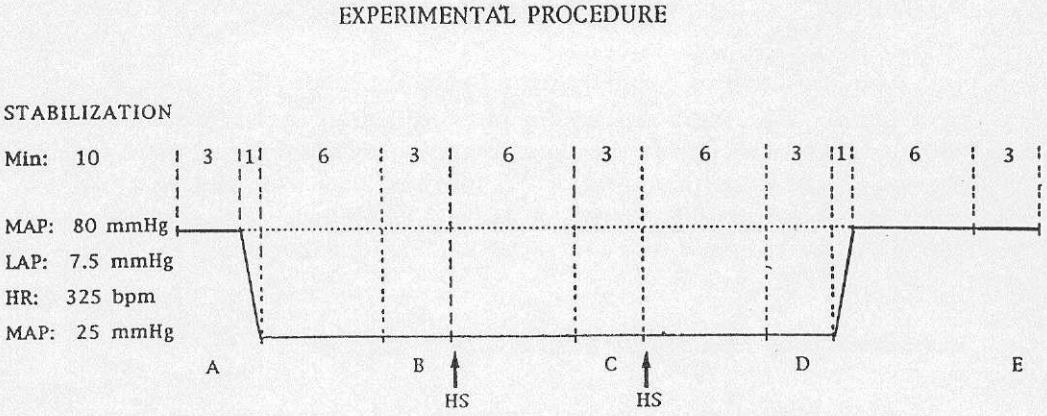

Figure 4. *Schematic drawing of the experimental procedure (paper II). HS denotes addition of hypertonic saline.*

Paper III:

After a stabilization period of 30 min baseline cardiodynamic and laser Doppler blood flow measurements were carried out and blood samples and muscle biopsies taken. Thereafter the balloon of the aortic catheter was inflated until pulsaltile back pressure disappeared and a subtotal ischaemia in both hind limbs achieved as evidenced by iliac back pressure and bilateral measurements of skin blood flow (laser Doppler). After 4 h of aortic occlusion the balloon was deflated and a 3 h reperfusion period was studied. Thereafter the animals were killed with i.v. KCl.

The animals were randomly assigned to three experimental fluid treatment groups. Fluid infusion was started 5 min prior to the release of the aortic occlusion and continued for 5 min after the reperfusion, i.e. the infusion was completed within a 10 min period. The following fluid treatment regimens were studied:

Group HS (n=8):

Hypertonic saline (7.5%; 2 400 mOsm/l) 4 ml/kg b.w.

Group HSD (n=8):

Hypertonic saline (HS 7.5%; 2 400 mOsm/l) in
6% Dextran 70 (Pharmacia, Stockholm, Sweden), 4 ml/kg b.w.

Group NS (n=8):

Normal saline 0.9% 4 ml/kg b.w.

Cardiodynamic and laser Doppler data were registered
- at baseline;
- at 1, 5 and 20 min after the aortic occlusion;
- thereafter every 20 min during the occlusion;
- 1, 5, 15 and 30 min after deflating the balloon;
- thereafter every 30 min.

Blood sampling and muscle biopsies were taken at baseline, after 220 min of ischaemia, 15 min after start of reperfusion (=10 min after the end of the treatment), and 180 min after start of reperfusion.

Paper IV:

The animals were randomly assigned to three experimental fluid treatment groups:

Group HS:
Hypertonic saline (7.5%; 2400 mOsm/kg), 4 ml/kg b.w.

Group HSD:
Hypertonic saline (HS 7.5%; in ***6% Dextran 70*** [Medisan Pharmaceuticals AB, Uppsala, Sweden]) 4 ml/kg b.w.

Group NS:
Normal saline 0.9%, 4 ml/kg b.w.

Fluid infusion was started 5 min prior to the release of the LAD-occlusion and continued for another 5 mins during reperfusion, i.e. the infusion was completed within a 10 min period.
The treatment fluids were administered intravenously into a peripheral vein and the fluid volumes infused were regulated by the use of a volume pump (IVAC Mod. No 561 MSS, IVAC Corporation, San Diego, California, U.S.)

Hemodynamic data were registered and blood chemistry analysed:
- at 10 min after finishing the catheterizations
  = baseline values before surgery.
- during a 30 min stabilisation period - at -30, -15, -5 min.
- during LAD-occlusion at +5, +15, +30, +40, +45 min.
- following reperfusion at 5, 15, 30, 60, 90, 120, 180, 240 min.

Blood sampling for serum markers of myocardial necrosis were obtained:

- at 10 min after completing the catheterizations
  = baseline values before surgery.
- during a 30 min stabilization period: -30 min, -5 min.
- during LAD-occlusion: +40 min.
- following reperfusion at 180 and 240 min.

At each blood sampling the withdrawn volume was compensated by an infusion of an equal volume of normal saline (drawn from 500 ml of 0.9% NaCl containing 5000 IU of heparin).

Statistics

Statistical comparisons between groups were performed with one-factor ANOVA with the Fisher`s protected least significant difference (PLSD) comparative test *(I, II, IV)* and two-factor repeated measures ANOVA *(I, III)*.

When significant ANOVA, the 2-tailed unpaired t-test *(II)* and the non-parametric analysis Mann-Whitney U-test *(IV)* was used to determine differences between two groups. In *paper III* the non-parametric analysis of variance, Kruskal-Wallis test was used to test differences between three groups. When significant, the Mann-Whitney U-test was used for statistical comparison between two groups. The 2-tailed student`s paired t - test *(I, II)* and the non-parametric Wilcoxon´s signed rank test *(I, III, IV)* was used to determine changes within a single group. Analysis of covariance was used to test for differences between groups when adjusted for baseline differences *(II)*. Regression analysis was used for comparison of the results from the inter-individual planimetric measurements for quantification of the infarct sizes and areas at risk *(IV)*.

In all studies data are presented as mean ± standard error of the mean (mean±SEM) and p-values of less than 0.05 are considered significant *(I-IV)*.

The computer software used for statistical analysis was StatView SE+Graphics (Abacus Concepts, Inc., Berkeley, CA, USA).

4. Results

Paper I

Changes in Blood Chemistry to Acute Blood Loss in SHR

Immediately after haemorrhage the *glucose* level had increased in the treatment groups ($p < 0.001$) and control group ($p < 0.01$). *Serum osmolality* had increased ($p < 0.05$) in the control and HG groups, while *PCV* had decreased ($p < 0.001$) from prehaemorrhagic values in all groups. At the end of bleeding the reduction of *PCV* was 12-15% in all groups. *Lactate* levels ($p < 0.001$) and pO_2 (NS- $p < 0.001$, C and HS- $p < 0.01$, HG- $p < 0.05$) increased and *base excess* (C, HS, NS- $p < 0.001$, HG- $p < 0.01$) and pCO_2 (C, HS, NS- $p < 0.001$, HG- $p < 0,05$) decreased similarly in all groups after bleeding.

Changes in Blood Chemistry 30 and 90 minutes after Treatment of Acute Blood Loss in SHR

At 30 mins after treatment *PCV* had been reduced from baseline levels by 20 % in controls, 22% with HS, 16% with HG, and 30 % with NS treatment. At 90 mins, haemodilution was of similar magnitude in HS- and NS-treated animals and greater than in the HG-treated animals ($p < 0.05$). *Sodium* levels were higher at 30 and 90 mins after HS treatment ($p < 0.05$) than in the other groups. *Serum osmoles* reached a maximum level of 310 ± 6.6 mOsm/kg water at 30 mins after HS (not significant vs other groups). At 30 mins, *glucose* levels were higher in the HG group ($p < 0.05$) than in the other groups. *Lactate* levels were at 30 mins lower in the NS group than in the HG group ($p < 0.05$), and after 90 mins lactate was lower in the HS and NS groups than in the HG group ($p < 0.05$). 30 mins after treatment pCO_2 was higher ($p < 0.05$) and the *base excess* lower ($p < 0.05$) in the NS group than the other groups, and at 90 mins this difference persisted for the NS group in comparison with the HG group. No significant *pH* changes were seen in any of the groups throughout the experiment.

Hemodynamic Responses to Acute Blood Loss in SHR

From prehaemorrhagic levels of a mean arterial pressure (MAP) of 175 ± 3 mm Hg and heart rate (HR) of 343 ± 8 beats per minute (bpm), MAP reached a level of 43 ± 3 mm Hg after haemorrhage of 30% of the calculated blood volume. In response to bleeding, increases as well as reductions of HR were seen in an inconsistent pattern. There were no differences in MAP and HR between groups before treatment.

Hemodynamic Responses after Treatment of Acute Blood Loss in SHR

Changes during 20 mins from start of treatment

The MAP response during the first 20-min period after start of treatment is detailed and compared with untreated animals in *Figure 5*. All treatment regimens raised MAP significantly ($p < 0.001$) vs the untreated control group (C). MAPs were higher after normal saline (NS) ($p < 0.001$) and hypertonic saline (HS) ($p < 0.01$) than after hypertonic glucose (HG), while there were no significant differences between HS and NS.

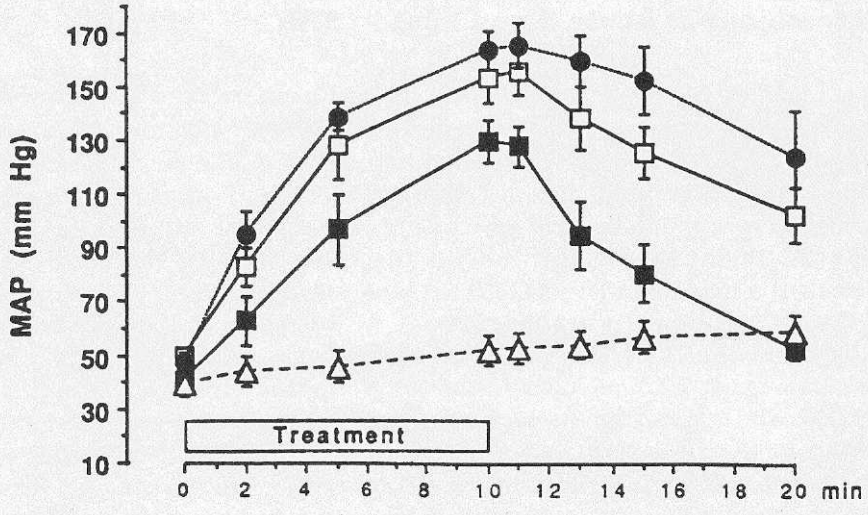

Figure 5. *MAP during 20 min after haemorrhage and ten min period of treatment with either hypertonic saline (HS), hypertonic glucose (HG), and normal saline (NS), and the control group (C).*

Changes during 160 mins from start of haemorrhage.

MAP and HR changes during 160 min period from start of haemorrhage are shown in the treatment groups in *Figure 6*. The control group is excluded in the figure because of its short survival time. Mortality in the treatment groups toward the end of this period were observed, and only 4 rats treated with HS, 5 with HG, and 7 with NS were alive.

MAP was higher after HS- ($p < 0.05$) and NS- ($p < 0.001$) than after HG- treatment, while there was no significant difference between HS and NS.

HR increased more in rats treated with HG ($p < 0.05$) and HS ($p < 0.01$) than in animals treated with NS.

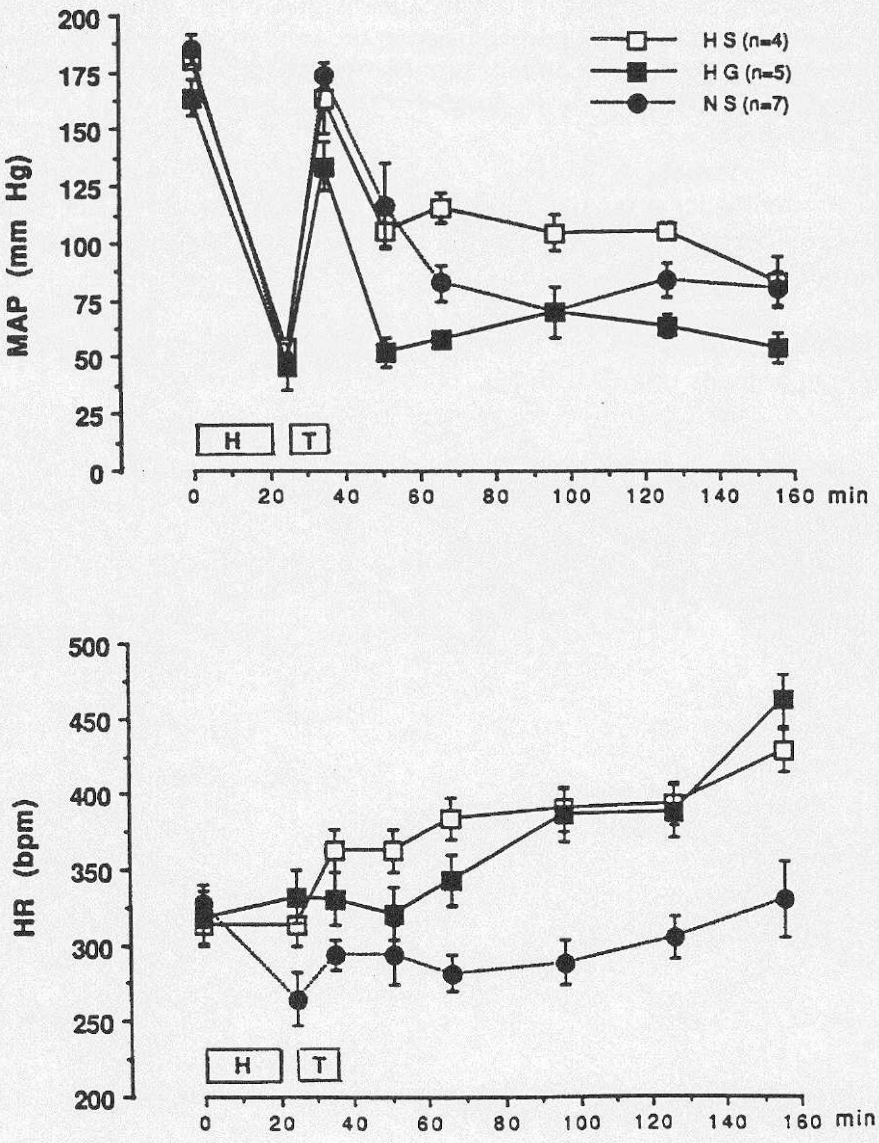

Figure 6. *MAP and HR in response to haemorrhage (H) and treatment (T) during 160 min period from start of haemorrhage.*

Times Until Death

No animals died before treatment was completed. The number of animals alive after the treatment period is shown in *Figure 7*. Mean times until death were 363 min (range 45-740 min) after treatment with NS, 170 min (32-455 min) after HS, 146 min (range 67-296 min) after HG, and 60 min (range 22-150 min) in untreated animals. Time until death was longer in all the treatment groups than in controls (NS vs C $p < 0.01$; HS and HG vs C $p < 0.05$), and longer for NS than for HG treated SHR ($p < 0.05$). No statistical significance in the HS group vs HG and NS.

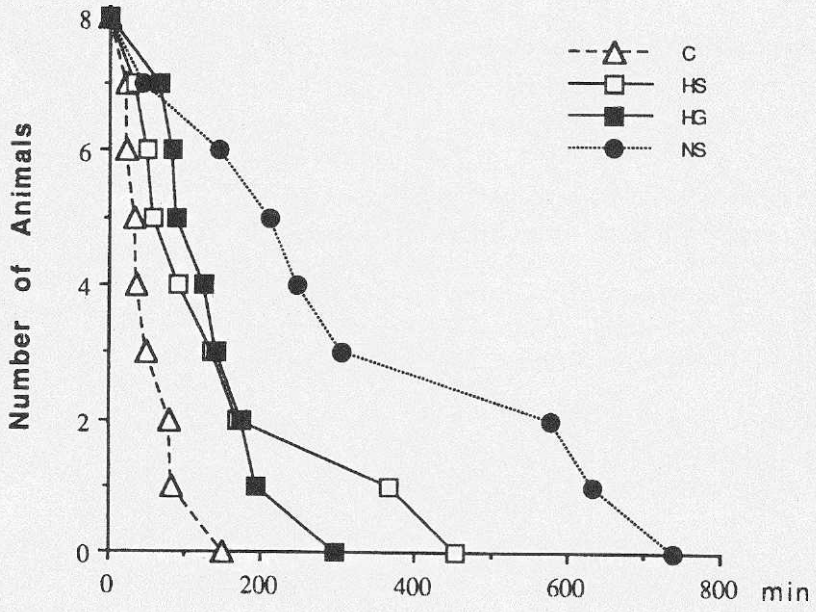

Figure 7. *Number of animals alive after the treatment period.*

Paper II

In this experimental study in ischaemic and nonischaemic isolated working rat hearts, the effects of hypertonic saline (HS) on myocardial function and metabolism were examined. Hypertonic saline administration to the perfusate caused an additional transient depression of the ischaemic hearts, and in the nonischaemic hearts a transient myocardial-depressant effect was also seen.

Initial concentrations (during period A) of sodium, potassium, and osmolality of the venous coronary effluent are shown in *Table 2*. Coronary flow (CF), stroke volume (SV), myocardial oxygen consumption (MVO_2), oxygen extraction (O_2-extr.), and lactate efflux in *Table 3*.

Myocardial Effects of Ischaemia

Control-Ischaemia group and Ischaemia plus Hypertonic Saline group

The myocardial ischaemia ensuing by the reduction of MAP to 25 mm Hg resulted in pronounced disturbances of cardiac function and metabolism. CF, SV, MVO_2, O_2-extraction and lactate efflux values were significantly ($p < 0.05$ to 0.001) altered compared with the nonischaemic group (control-hypertonic saline group; control period B through D; *Figures 8 and 9, Table 3*).

Sodium, Potassium, and Osmolality of the Perfusate after Hypertonic Saline

Ischaemia Plus Hypertonic Saline group and Control-Hypertonic Saline group

Administration of hypertonic saline resulted in a similar increase of sodium concentration in the perfusate in the ischaemia plus hypertonic saline and the control-hypertonic saline groups, while the potassium concentration remained unchanged and similar in all three groups. The osmolality increased by ~30 mOsm/kg after hypertonic saline in both groups *(Table 2)*.

Table 2. Sodium, Potassium, and Osmolality during a Low Flow Global Ischaemia, and Administration of Hypertonic Saline 3 % (mean ± SEM)

	A	B	C	D	E
Sodium (mmol / l)					
C - I	139.6 ± 0.9	139.9 ± 0.8	139.6 ± 0.9	140.1 ± 1.3	140.1 ± 0.6
I + HS	137.8 ± 0.8	136.9 ± 0.5	151.1 ± 0.5[a]	157.9 ± 0.8[a]	160.6 ± 0.8[a]
C - HS	140.0 ± 1.2	140.0 ± 0.9	155.5 ± 1.0[a]	162.8 ± 1.4[a]	162.9 ± 1.5[a]
Potassium (mmol / l)					
C - I	5.55 ± 0.04	5.56 ± 0.03	5.56 ± 0.04	5.58 ± 0.05	5.58 ± 0.03
I + HS	5.45 ± 0.03	5.46 ± 0.03	5.50 ± 0.03	5.49 ± 0.03	5.53 ± 0.02
C - HS	5.60 ± 0.05	5.60 ± 0.04	5.60 ± 0.04	5.58 ± 0.04	5.60 ± 0.04
Osmolality (mOsm / kg)					
C - I	300.6 ± 1.2	302.8 ± 1.6	303.9 ± 1.6	302.6 ± 1.3	303.3 ± 1.6
I + HS	297.7 ± 1.6	302.3 ± 1.8	329.1 ± 1.7[a]	349.3 ± 5.3[a]	345.4 ± 1.7[a]
C - HS	305.8 ± 2.6	304.8 ± 2.4[b]	335.3 ± 2.7[a]	349.0 ± 3.0[a]	350.6 ± 3.5[a]

A, baseline control period; B,C,D, ischaemic control periods (control periods in the control-hypertonic saline group); E = postischaemic control period in the control-ischaemia and ischaemia plus hypertonic saline groups (control period in the control-hypertonic saline group). C-I, control-ischaemia; I+HS, ischaemia plus hypertonic saline; C-HS, control plus hypertonic saline. I+HS, C-HS vs C-I [a] $p < 0.001$; C-HS vs I+HS [b] $p < 0.05$.
All p -values refer to differences from baseline between groups.

Table 3. Myocardial Circulatory and Metabolic Effects of Hypertonic Saline during Low-Flow Global Ischaemia (mean ± SEM)

	A	B	B_1	B_3	C	C_1	C_3	D	E
Coronary Flow (ml / min / g heart weight)									
C - I	14.7 ± 1.0	2.4 ± 0.6[a]	2.4 ± 0.6[a]	2.4 ± 0.6[a]	2.4 ± 0.6[a]	2.4 ± 0.6[a]	2.4 ± 0.6[a]	2.4 ± 0.6[a]	14.0 ± 1.1
I + HS	14.9 ± 1.2	2.0 ± 0.2[a]	2.0 ± 0.2[a]	2.2 ± 0.2[a]	2.0 ± 0.2[a]	1.8 ± 0.3[a]	2.0 ± 0.3[a]	2.1 ± 0.2[a]	13.1 ± 0.7
C - HS	14.9 ± 0.6	14.3 ± 0.4	12.0 ± 0.4	13.8 ± 0.6	14.2 ± 0.5	12.5 ± 0.3	13.9 ± 0.6	14.2 ± 0.5	13.4 ± 0.6[g]
Stroke Volume (µl / 100 g body weight)									
C - I	49.9 ± 1.8[c]	19.9 ± 0.8[a]	19.3 ± 0.6[a]	19.6 ± 0.6[a]	19.8 ± 0.7[a]	19.4 ± 0.7[a]	19.5 ± 0.9[a]	19.6 ± 0.5[a]	46.0 ± 2.7
I + HS	59.8 ± 5.0	25.8 ± 2.1[ad]	15.3 ± 0.9[ae]	25.7 ± 2.0[ad]	25.6 ± 1.8[ad]	19.1 ± 1.7[a]	23.6 ± 2.2[a]	23.4 ± 2.4[a]	45.2 ± 3.6[f]
C - HS	60.7 ± 2.8	60.4 ± 2.6	43.5 ± 2.0	58.1 ± 2.4	56.5 ± 2.6	46.1 ± 2.5	52.3 ± 2.8	53.4 ± 2.4	48.4 ± 2.4[f]
Myocardial Oxygen Consumption (mmol / min / g heart weight)									
C - I	4.7 ± 0.4	0.8 ± 0.1[a]	0.8 ± 0.1[a]	0.9 ± 0.1[a]	0.9 ± 0.1[a]	0.8 ± 0.1[a]	0.8 ± 0.1[a]	0.8 ± 0.1[a]	4.7 ± 0.5
I + HS	5.7 ± 0.5	0.8 ± 0.1[a]	0.8 ± 0.1[a]	1.0 ± 0.1[a]	0.8 ± 0.1[a]	0.8 ± 0.1[a]	0.8 ± 0.1[a]	0.9 ± 0.1[a]	5.2 ± 0.3
C - HS	5.5 ± 0.3	5.4 ± 0.3	4.4 ± 0.2	5.1 ± 0.3	5.3 ± 0.2	4.6 ± 0.2	5.2 ± 0.3	5.3 ± 0.3	5.1 ± 0.2[g]
Myocardial Oxygen Extraction (%)									
C - I	71.7 ± 3.5	87.0 ± 6.1[b]	86.3 ± 6.7[b]	87.9 ± 5.6[b]	87.5 ± 5.9[b]	87.9 ± 5.8[b]	87.9 ± 5.9[b]	88.3 ± 5.6[b]	75.1 ± 4.6[g]
I + HS	81.9 ± 3.7	91.0 ± 2.6[a]	90.9 ± 2.7[a]	90.7 ± 2.7[a]	90.8 ± 2.7[a]	90.9 ± 2.7[a]	90.6 ± 2.7[a]	91.0 ± 2.7[a]	83.0 ± 3.1
C - HS	73.9 ± 2.3	75.1 ± 2.1	72.4 ± 2.0	73.3 ± 2.4	74.4 ± 2.2	73.5 ± 2.1	74.1 ± 2.2	74.2 ± 2.3	75.4 ± 2.3
Lactate efflux (µmol / min / g heart weight)									
C - I	1.6 ± 0.4	2.3 ± 0.3[c]	-	-	2.5 ± 0.3[c]	-	-	2.4 ± 0.4[b]	1.4 ± 0.3
I + HS	1.5 ± 0.2	3.2 ± 0.3[a]	-	-	3.3 ± 0.3[a]	-	-	3.4 ± 0.3[ad]	1.8 ± 0.2
C - HS	1.3 ± 0.2	1.4 ± 0.2	-	-	1.4 ± 0.2	-	-	1.3 ± 0.2	1.4 ± 0.2

A, baseline control period; B,C,D, ischaemic control periods (control periods in the control-hypertonic saline group); B_1, C_1,1 min after administration of hypertonic saline; B_3, C_3, 3 mins after administration of hypertonic saline in the ischaemia plus hypertonic saline and control-hypertonic saline groups, respectively; E = postischaemic control period in the control-ischaemia and ischaemia plus hypertonic saline groups (control period in the control-hypertonic saline group); C-I, control-ischaemia; I+HS, ischaemia plus hypertonic saline; C-HS, control-hypertonic saline. C-I, I+HS vs C-HS [a] $p < 0.001$; [b] $p < 0.05$; [c] $p < 0.01$; I+HS vs C-I [d] $p < 0.05$; [e] $p < 0.01$. [f] $p < 0.001$; [g] $p < 0.05$ period E compared with baseline A.

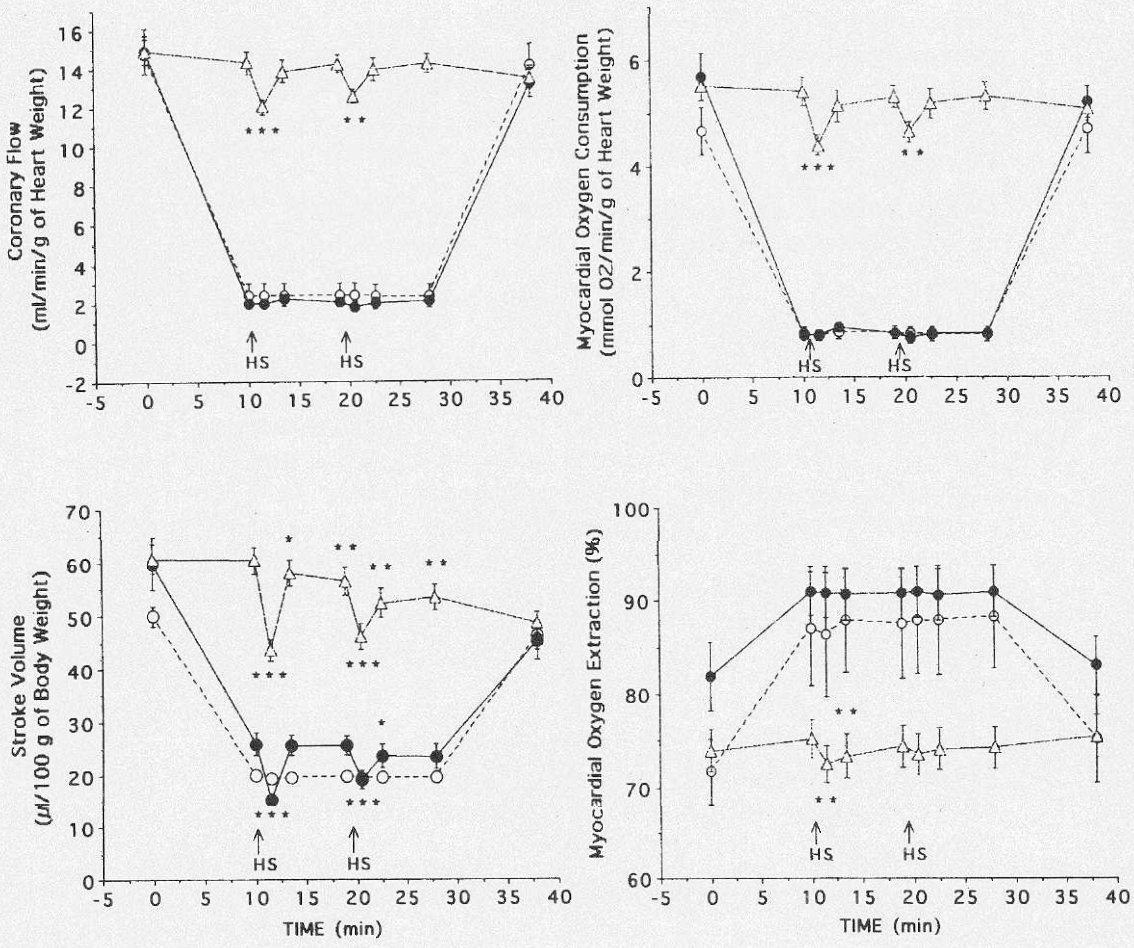

Figure 8. *Effects of repeated administration of hypertonic saline (HS) on coronary flow, stroke volume, myocardial oxygen consumption, and myocardial oxygen extraction. Open circles, control hearts receiving ischaemia; solid circles, hearts receiving ischaemia and hypertonic saline; open triangles, control hearts receiving hypertonic saline. Values are mean ± SEM. Statistically significant differences are denoted for effects of hypertonic saline: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with values before the latest HS administration.*

After the second administration of HS a further increase in sodium concentration to ~160 mmol/l from ~150 mmol/l was measured, while potassium remained mainly unchanged. The osmolality increased similarily in both groups reaching values of ~350 mOsm/kg. The sodium concentration and osmolality remained at this high level in the postischaemic period (E).

Myocardial Functional and Metabolic Effects of Hypertonic Saline in Ischaemic Hearts

Ischaemia plus Hypertonic Saline group

CF in the ischaemic hearts was not influenced by hypertonic saline, *(Figure 8, Table 3)*, while SV decreased transiently within 1 min (B to B_1 [1 min after the initial administration of HS] and C to C1 [1 min after the second administration of HS]) after both HS administrations ($p < 0.001$). SV returned within 3 mins to the preinfusion value after the first infusion. However, after the second infusion SV remained significantly ($p < 0.05$) depressed for a long period of time *(Figure 8, Table 3)*. HS was not found to have significantly influenced MVO_2 and O_2-extraction, *(Figure 8, Table 3)*, or lactate efflux *(Table 3)*.

Myocardial Functional and Metabolic Effects of Hypertonic Saline in Nonischaemic Hearts

Control-Hypertonic Saline group

CF decreased significantly after both HS administrations within 1 min (B to B_1 [$p < 0.001$]; C to C_1 [$P < 0.01$]; *(Figure 8, Table 3)*. The reductions were transient, and CF returned to previous values within 3 mins. SV decreased significantly ($p < 0.001$) after both HS administrations within 1 min (B to B_1; C to C_1; *Figure 8, Table 3*) and did not return to previous values within 3 mins (B to B_3 [$p < 0.05$]; C to C_3 [$p < 0.01$]).

HS caused a transient decrease in MVO_2 1 min after the first (B to B_1 [$p < 0.001$]) as well as after the second HS administration (C to C_1 [$p < 0.01$]). However, MVO_2 returned to the previous values within 3 mins *(Figure 8, Table 3)*. O_2-extraction decreased slightly but significantly 1 min after the first HS administration (B to B_1 [$p < 0.01$]).

The recovery to value before the administration of HS was somewhat slow and the difference between the pre-administration value and the value 3 mins after administration of HS was still significant ($p < 0.01$), but in period C, O_2-extraction had returned to its previous value *(Figure 8, Table 3)*. After the second HS administration O_2-extraction did not change significantly. HS was not found to influence the lactate efflux of the nonischaemic isolated heart *(Table 3)*.

Change in Percent over Time from Baseline Values

Comparisons of myocardial function and metabolism over time (periods A vs E) in the three different experimental groups indicate significant reductions of SV in the HS groups ($p < 0.001$) with 24.4 % in the *ischaemia plus hypertonic saline group* and with 20.3 % in the *control-hypertonic saline group*. The reductions differed significantly ($p < 0.05$) from the *control-ischaemia group* (7.8 %), while CF, O_2-extraction, MVO_2 and lactate efflux values did not differ between groups *(Figure 9)*.

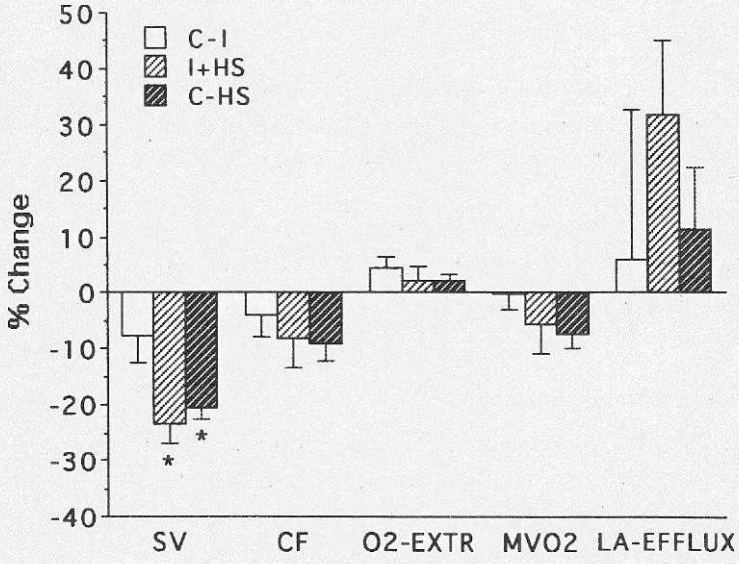

Figure 9. *Percent change from baseline to end of experiment of stroke volume (SV), coronary flow (CF), myocardial oxygen extraction (O_2 Extr.), myocardial oxygen consumption (MVO_2), and cardiac lactate efflux (LA Efflux). Open bars, control hearts receiving ischaemia; lightly hatched bars, hearts receiving ischaemia and hypertonic saline. darkly hatched bars, control hearts receiving hypertonic saline. Values are mean ± SEM. * $p < 0.05$ vs control animals receiving ischaemia.*

Paper III

This study was focused on the effects of hypertonic saline and hypertonic saline/dextran on central haemodynamics, leg blood flow, and skeletal muscle metabolism at reperfusion after subtotal bilateral limb ischaemia.

Effects of Distal Aortic Occlusion

Central haemodynamics

The aortic occlusion resulted in a rapid initial increase of MAP ($p < 0.0001$) and SVR ($p < 0.001$) while HR ($p < 0.0001$) slowly increased over time. The CVP ($p < 0.01$), SVI ($p < 0.001$), iliac back pressure (IBP) ($p < 0.0001$), and skin blood flow (SBF) ($p < 0.0001$) were reduced. Cardiac index was unchanged.

Blood Chemistry

Serum electrolytes, osmolality, haemoglobin, haematocrit, and total protein levels remained mainly unchanged (ns) although there was a slight increase of the serum potassium level ($p < 0.05$).

Acid base data showed a slightly increased pH level ($p < 0.05$), and reduced P_aCO2 ($p < 0.05$), P_aO_2 ($p < 0.05$), and O_2-saturation ($p < 0.05$), while base excess remained mainly unchanged (ns).

Skeletal Muscle Metabolism

The skeletal muscle content of the high energy phosphagens ATP ($p < 0.05$) and CrP ($p < 0.0001$) was reduced and the lactate content increased ($p < 0.001$) at 220 min of aortic occlusion.

Between Groups Differences during distal aortic occlusion

There were no significant differences in the overall response pattern to aortic occlusion between the different treatment groups although there were slightly lower levels of sodium and osmolality in the HS group vs the NS group ($p<0.05$).

Fluid Therapy, Reperfusion Characteristics, and Biochemical Alterations

Central haemodynamics

During the 5-min period of fluid infusion prior to aortic reflow, MAP increased significantly in the HS and HSD groups ($p < 0.05$) while no major change was seen in the NS group. The release of the aortic occlusion resulted in a reduction of MAP in all groups ($p < 0.05$). MAP remained lower in the NS than in the HS and HSD groups ($p < 0.05$-0.01 vs NS) during the reperfusion period *(Figure 10)*.

The blood pressure in the iliac artery (IBP) increased at reflow in all groups with the most pronounced increase in the HS group *(Figure 10)*.

HR was slightly reduced in the NS and HSD groups while HR increased ($p < 0.05$) in the HS group during the initial 5 min treatment period significantly ($p < 0.01$) vs the NS group. HR increased in all groups during reperfusion.

CI increased ($p < 0.05$) in the HS and HSD groups at the initial fluid infusion while the changes in SVI remained non-significant. Following reperfusion a drop of CI and SVI occurred in all groups. There were no significant differences between the groups in CI and SVI.

CVP increased more in the HS and HSD groups ($p < 0.05$) than in the NS group during the fluid infusion prior to aortic reflow *(Figure 11)*.

The thoracic fluid index (TFI) values increased ($p < 0.05$) in the NS group at 15 min after reperfusion and remained increased while in the HS and HSD groups TFI was significantly reduced throughout the whole reperfusion period *(Figure 11)*.

SVR was reduced immediately following reflow in all groups and during prolonged reflow no significant differences between the groups were demonstrable.

Peripheral skin perfusion (Laser doppler) increased following reperfusion to values near preocclusion baseline levels in all groups.

There was a calculated blood loss of about 4 ml/kg b.w. in all groups. Urine output remained at about 30 ml/h throughout the experimental period. There were no differences beween the groups in body weight changes during the experiment.

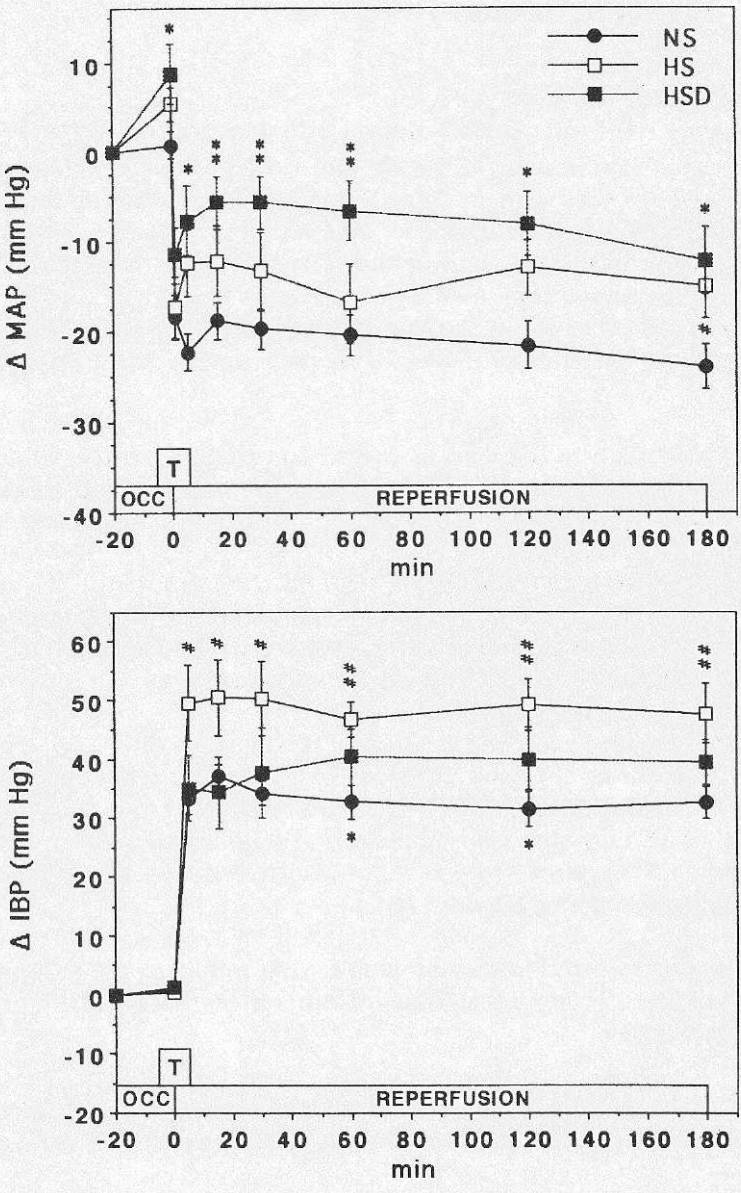

Figure 10. *Effects of fluid therapy on mean arterial pressure (MAP) and iliac artery blood pressure (IBP) prior to and after deflation of aortic balloon.* $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ *HS vs NS;* $^{*}p < 0.05$; $^{**}p < 0.01$ *HSD vs NS. OCC = occlusion; T = fluid treatment period. Values are mean ± SEM.*

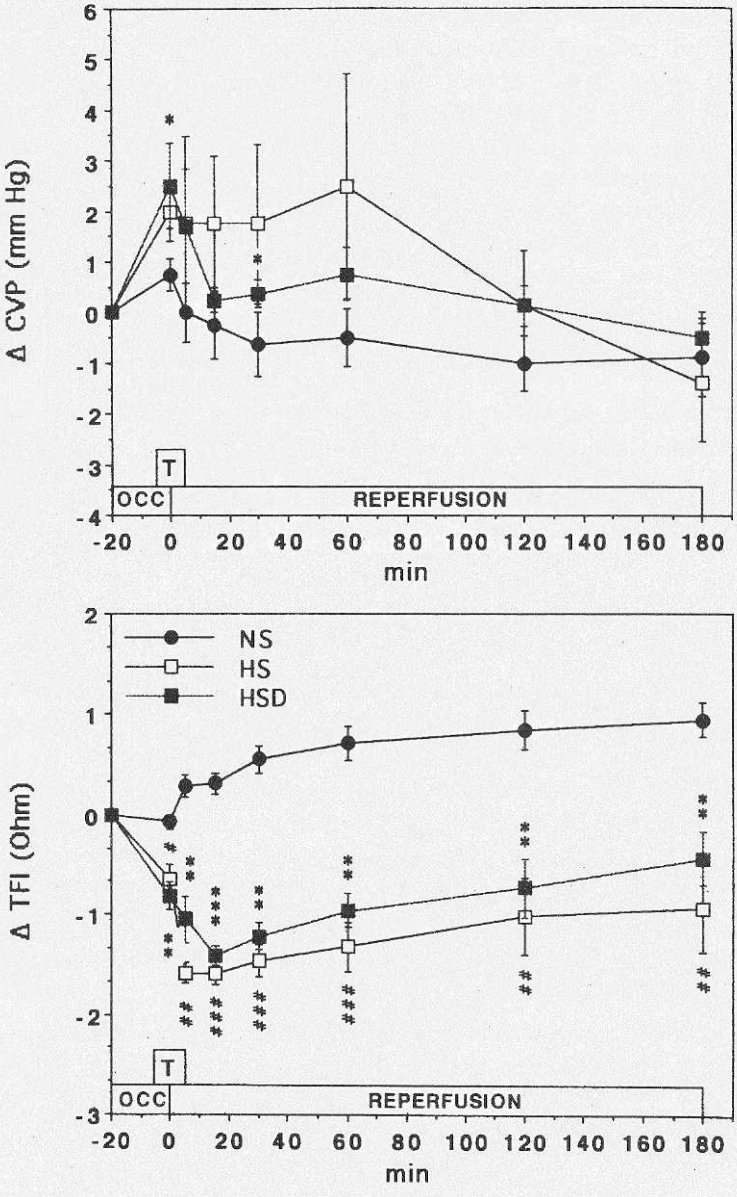

Figure 11. *Effects of fluid therapy on central venous pressure (CVP) and thoracic fluid index (TFI) prior to and after deflation of aortic balloon.* $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ *HS vs NS;* $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ *HSD vs NS. OCC = occlusion; T = fluid treatment period. Values are mean ± SEM.*

Blood Chemistry

Serum sodium remained unchanged in the NS group while the infusions of HS and HSD resulted in S-Na and S-Cl increments of about 10 mmol/l ($p < 0.05$). The corresponding increase in serum osmolality in the HS and HSD groups was about 20 mOsm/kg ($p < 0.05$). S-K increased in all groups in the post-ischaemic period ($p < 0.05$). Haemoglobin and haematocrit increased in the NS group ($p < 0.05$) while serum protein levels were reduced in the HS and HSD groups ($p < 0.05$) at 15 min following the initial fluid treatment and reflow. At 180 min no significant differences in haemoglobin, haematocrit or serum protein levels remained between the different groups.

Acid-base values were only marginally altered during the experimental procedure. At 15 min following reflow pH was reduced by 0.07-0.10 pH units and base excess values by about 3 mmol/l in all groups. After 180 min of reflow the acid-base status had normalised in all groups and no inter-group differences were demonstrable.

Skeletal Muscle Metabolism

A significant regain of high energy phosphagens (ATP+CrP) 15 min after reflow was observed in the HS group ($p < 0.05$). At 180 min after reperfusion no significant differences between the treatment groups remained *(Figure 12)*. The initial reduction of the lactate content of skeletal muscle at 15 min was more pronounced ($p < 0.05$) in the HSD than in the HS and NS groups. Further reduction of the lactate levels were seen after 180 min in all treatment groups *(Figure 12)*.

Skeletal Muscle Water Content

The water content of skeletal muscle was significantly increased ($p < 0.05$) in the NS group at 180 min following reflow but not in the HS or HSD groups.

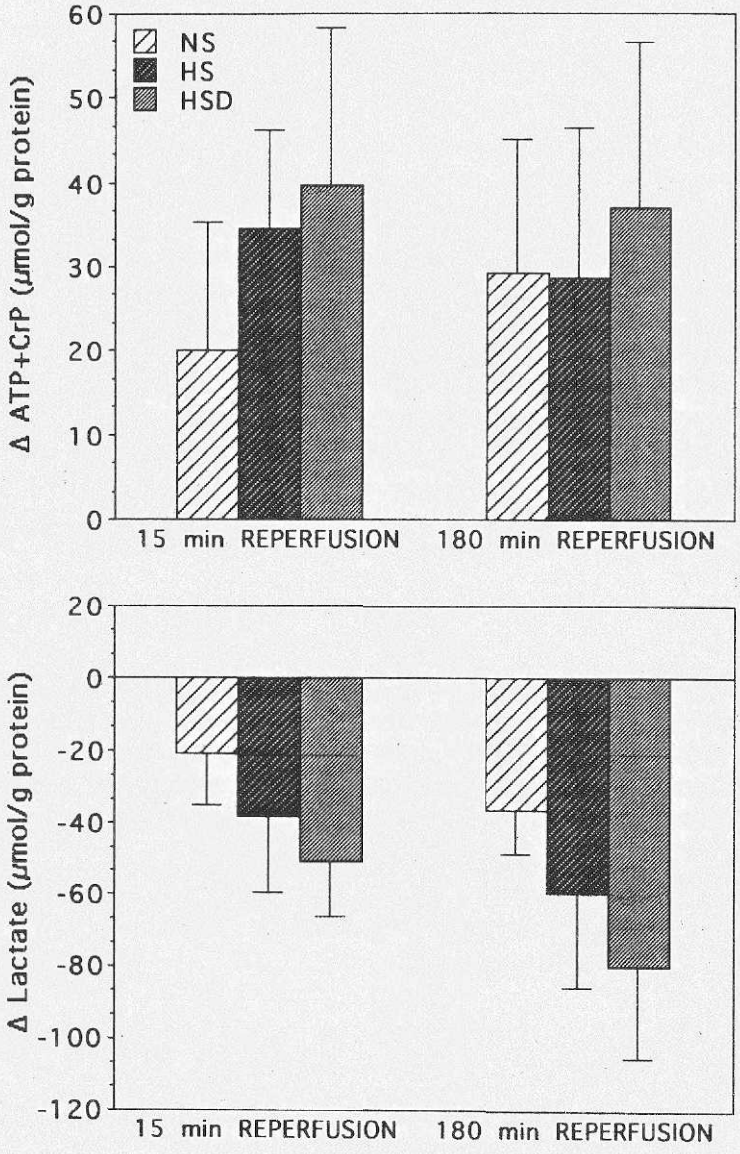

Figure 12. *Regain of high energy phosphagens and wash out of lactate in skeletal muscle 15 min and 180 min following fluid treatment and reperfusion. Values are ± SEM.*

Paper IV

This study investigates if hypertonic saline ± dextran is safe as volume loading fluid in hypovolaemic situations (sepsis, burns, and haemorrhage due to surgery or trauma) for individuals with depressed left ventricular function. The study is following up paper II, where the direct effects of hypertonic saline on myocardial function and metabolism was investigated in isolated ischaemic and nonischaemic hearts.

Randomization and Inclusion of the Animals

In total 38 animals were randomized into the study (HS, n=12; HSD, n=13; NS, n=13). Three animals in the HSD group were excluded due to ventricular fibrillation resistant to defibrillation (at 15, 23, 23 min after start of reperfusion) and one animal in the NS group due to basal signs of myocardial damage (high pre-occlusion Troponin-T value of 4.65 μg/l). Another four animals developed ventricular fibrillation (HS, n=2 at 1 and 5 min following reperfusion; HSD, n=1 at 5 min following onset of occlusion; NS, n=1 at 30 min following onset of occlusion) but could all be successfully defibrillated within < 2 min without remaining circulatory disturbances and were therefore included in the data analysis. The presented data are based on 34 animals (HS, n=12; HSD, n=10; NS, n=12).

Effects of Catheterization, Preparative Procedures, and 40-min of LAD Occlusion

Blood chemistry variables were at 10 min after catheterization within the normal range for the pig in all groups and the haemodynamics did not differ significantly. After the preparative procedures and the 30 min period of stabilisation there were some changes of minor significance.

Occlusion blood chemistry data

There were only minor changes in serum electrolytes, osmolality, total protein, glucose, blood lactate, and haematocrit levels during the LAD occlusion period as well as for acid-base and oxygenation data.

Occlusion haemodynamic data

The myocardial ischaemia caused by the LAD occlusion resulted in a general decrease in MAP, CI, SVI, LVSWI, LVP, and +dP/dt. HR increased in the HSD and NS groups, and decreased slightly in the HS. PCWP, LVEDP, and PVR increased in all groups. Between the three experimental groups there were no significant differences in the haemodynamic changes during the LAD occlusion.

Effects of Volume Loading and Reperfusion

Blood chemistry

The 10-min period of infusion (from 5-min before to 5 min after release of occlusion) sodium increased significantly reaching 152 mmol/l ($p < 0.01$) in the HS and HSD ($p < 0.001$ HS and HSD vs NS) groups while in the NS group sodium remained unchanged. Chloride which increased in the HS and HSD groups ($p < 0.001$) was unchanged in the NS group ($p < 0.001$ HS and HSD vs NS). Na^+ and Cl^- remained significantly elevated in the HS and HSD groups ($p < 0.001$ vs NS) throughout the experiment until 240 min of reperfusion. Corresponding dilution-associated decreases of K^+ and Ca^{++} were initial seen in the HS and HSD groups ($p < 0.01$-0.001 HS and HSD vs NS). At later stages after the reperfusion K^+ increased in all groups to levels at about 5 mmol/l.

Osmolality increased significantly ($p < 0.01$) by 22 mOsm/kg in both HS and HSD groups at the end of the volume loading and remained increased in comparison to the NS group ($p < 0.001$) throughout the 240 min reperfusion study period.

Total protein ($p < 0.01$-0.001 HS and HSD vs NS), blood glucose (ns HS and HSD vs NS), and haematocrit ($p < 0.05$-0.001 HS and HSD vs NS) decreased in response to the initial volume loading in all groups but significantly more in the HS and HSD groups. After 240 min of reperfusion the decrease in total protein and haematocrit were still significant in the HS and HSD groups vs NS ($p < 0.01$).

Blood lactate decreased significantly in all groups with no difference between the groups.

Acid base and oxygenation data

The effects of volume loading and initial reperfusion on acid base and oxygenation data were only minor. In the HS and HSD groups pH ($p < 0.05$ HS vs NS; 0.05-0.01 HSD vs NS), base excess ($p < 0.01$ HSD vs NS), and $avDO_2$ ($p < 0.01$ HS and HSD vs NS) values decreased somewhat while PaO_2, SaO_2, SvO_2, and $PaCO_2$ remained mainly unchanged in all groups. At 240 min of reperfusion DO_2 and VO_2 were reduced in all groups as part of a general haemodynamic deterioration at that stage. Acid base values were at this stage still mainly unchanged at the preocclusion level.

Haemodynamics

No significant effects of the different treatment fluids on HR and RPP were observed.

MAP and MPAP were in the HSD group initially increased vs the NS group ($p < 0.05$, $p < 0.01$) and at 15 mins reperfusion the increase in MPAP was also significantly higher vs HS ($p < 0.01$).

CVP and PCWP increased initially more in the HS and HSD groups than in the NS group ($p < 0.001$-0.05 vs NS), and CVP remained significantly elevated in the HS group vs NS throughout the 240 min reperfusion period. PCWP was significantly elevated in the HSD group during 90 mins ($p < 0.001$-0.05), and in the HS group ($p < 0.001$-0.05) vs the NS group during 120 mins of the reperfusion period.

Initially CI increased significantly vs NS in the HS and HSD groups ($p < 0.05$), and SVI increased only in the HSD group ($p < 0.05$-0.01 vs NS, and $p < 0.05$ vs HS).

LVEDP increased initially more in the HS and HSD groups ($p < 0.05$ vs NS).

LVSWI was significantly increased at 30 and 60 mins of reperfusion in the HSD group vs HS ($p < 0.05$).

+dP/dt was unchanged (HSD) or depressed (HS) ($p < 0.01$-0.05 vs NS from15 min to 120 min during the reperfusion period by the hyperosmolar fluid treatment.

SVR and PVR were initially reduced by HS and HSD vs NS ($p < 0.05$).

The duration of the volume expansion was more pronounced by HSD than by HS and NS infusion indicated by the LVSWI and LVEDP relationships.

Release of Serum Markers of Myocardial Necrosis and Ischaemia

During the surgical procedure, the isolation of LAD, and LAD occlusion only minor changes of the serum levels of ASAT, CK, CKMB, and TNT occurred and blood lactate was slightly increased by about 0.6 mmol/l.

At 180 min following reflow after the LAD occlusion period the serum levels of these markers of myocardial damage were all elevated and remained increased throughout the studied 240 min period. In the HSD group the increase of ASAT was more pronounced ($p < 0.05$ vs NS and HS at 180 min reperfusion, $p < 0.01$ vs HS at 240 min reperfusion) than in the HS and NS groups but no differences in CK, CKMB, and TNT between the different groups were observed.

Myocardial Muscle Metabolites

No differences related to the fluid treatment strategy chosen were demonstrable in the tissue levels of high energy phosphagens in the ischaemic myocardium at the end of the experiment *Figure 13.*

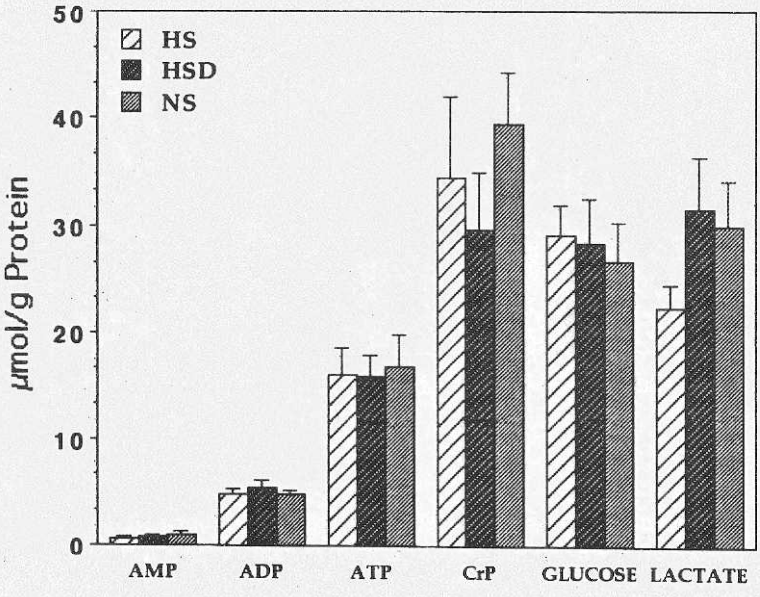

Figure 13. *Myocardial muscle metabolites. Adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP), creatine phosphate (CrP), glucose, and lactate.*

Area at Risk and Infarct Size Quantification

The results of the infarct and risk area quantifications are given in *Figure 14*. The area at risk given as percentage of the left ventricle and the infarcted area, given as percentage of the area at risk, were in the HS group 32.4±2.9 % and 20.2±3.3 % respectively. The corresponding values for the HSD group were 26.9±3.4 % and 23.7±3.9 %, and for the NS group 29.3±2.5 % and 22.7±4.3 %. The calculated infarcted areas in percentage of the left ventricle were for HS 6.1%, for HSD 6.6 %, and for NS 6.5 %. There were no significant differences in the quantified areas of myocardial injury between the experimental groups.

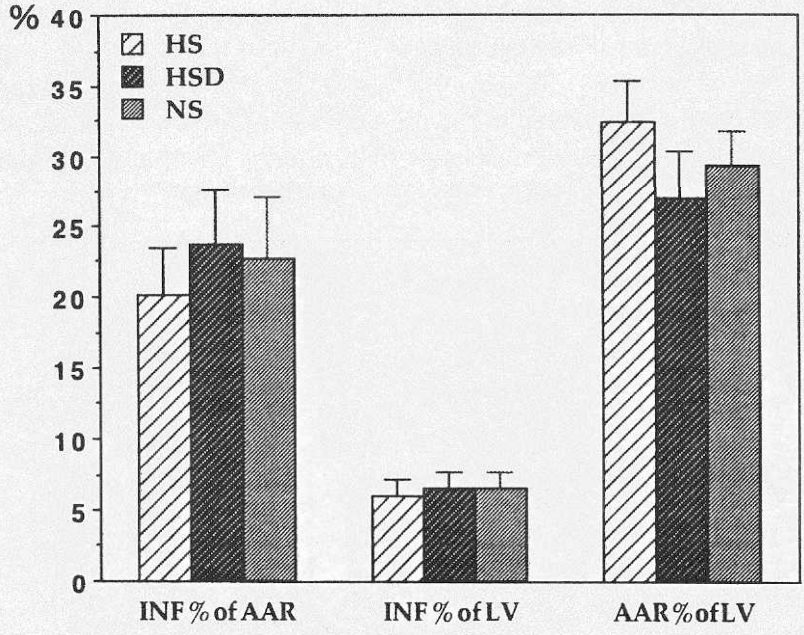

Figure 14. *Area at risk and myocardial infarct size. Myocardial infarction in % of area at risk (INF % of AAR), myocardial infarction in % of left ventricle (INF % of LV), and area at risk in % of left ventricle (AAR of LV).*

5. Methodological and Experimental Considerations

Experimental Models

The acute blood loss of 30% of estimated blood volume in the SHR in *paper I*, resulting in a MAP decrease from ~175 to 43 mm Hg. The prehaemorrhagic MAP level of SHR in the present study was similar to that previously observed for anesthetized SHR[57-59] indicating a representative state of hypertension at induction of haemorrhage. Because of the simultaneously increased mortality and decreased ability to maintain hyperglycaemia after haemorrhage in the SHR,[57,60] SHR was used for evaluation of the efficiency of hypertonic treatment.

In *paper II* the aim was to study direct effects of hypertonic saline on the function of nonischaemic and ischaemic myocardium, perfusion, and metabolism. The model used was an isolated, anterogradely perfused, atrial paced working rat heart preparation. The pacing at constant heart rate and adjustment of pre- and afterload to predetermined levels makes the maintenance of standardized physiological conditions possible. This model allows assessment of the effects on myocardial function, perfusion, and metabolism at constant afterload, preload, and heart rate in rats after exposure to standardized, low-flow, cardiac ischaemia.[49,61] During the experimental procedure there was no deterioration in myocardial function. This is evidenced by the fact that the myocardial function and metabolism (stroke volume, coronary flow, O_2-extraction, MVO_2, and LA-efflux) of control hearts after exposure to ischaemia returned to initial baseline values at the end of the experiment *(Figure 9)*.

In order to study physiological events in acute limb ischaemia, an atraumatic model simulating the clinical situation is ideal. Many experimental models for acute limb ischaemia include extensive dissection or use of tourniquets causing local pressure and sometimes

also venous obstruction. The model used in *paper III* is a relatively atraumatic pig model for acute bilateral hind limb ischaemia, simulating acute aortic saddle embolism. This previously described experimental model for simulation of clinical acute aortic saddle embolism was considered to constitute a suitable model for assessing the influences of different fluid treatment regimens on the post-ischaemic recovery of tissue perfusion and metabolism.[51]

In *paper IV* an experimental porcine model of LAD occlusion and reperfusion was chosen because of similar physiology to humans as well as lack of collateral coronary circulation.[62-65]
The model was similar to that described by Hamburger et al,[52] with the exception of the anaesthetic technique used.

Anaesthesia and Treatment Fluids

Anaesthesia *(I, II)*

Depending on the need of duration for anaesthesia a short-acting barbiturate, pentobarbital was chosen in *paper I* and a more ultra-short-acting barbiturate, metohexital in *paper II*.

Barbiturates as well as all general anaesthetic agents studied cause myocardial depression at concentrations that are clinically or experimentally useful. This is a result of both by decreasing Ca^{2+} influx across the plasma membrane and reduced calcium ion concentrations in the sarcoplasmatic reticulum.[66,67] During pacing of Langendorff type isolated heart preparation, left ventricular mechanical performance and O_2 consumption was not affected by pentobarbital. Pentobarbital treated hearts were also completely insensitive to three times increases of Ca^{2+} in the perfusate.[68] The same is probably true also for metohexital and this agent is also rapidly eliminated .

Anaesthesia *(III, IV)*

For induction of anaesthesia in *paper III* and *IV* we used the rapid-acting analgesic and dissociative anaesthetic agent, ketamine (Ketalar®), and the anaesthesia was maintained with the hypnotic agent methomidate (Hypnodil®) and the sedative agent azaperone (Stresnil®).

Anaesthesia with Methomidate and Azaperone demonstrates a marked degree of cardiovascular stability during and after anaesthesia. The heart, and respiratory rate slow down, probably because of lowered oxygen needs (Janssen Pharmaceutica, Beerse, Belgien).

Ketamine has successfully been used in cardiac surgery in pigs,[65] and is perhaps the most widely and safety used drug in porcine studies. Ketamine has marked effects on the cardiovascular system. It stimulates the heart in a clinically normal subject, manifested as an increase in heart rate, mean arterial pressure and cardiac output. Theese are probably indirect effects caused by a combination of parasympathetic inhibition and sympathetic stimulation.[69] Subjects with cardiac arrhythmias do not appear to be detrimentally affected, but extreme caution is recommended in those with diseased or traumatized myocardium due to a direct negative inotropic effect coupled with an increase in cardiac work. The peripheral vasopressor response is considered to be centrally mediated, and there is no reported increase in preload.[69-71] The stimuatory effects could be attenuated by co-administration of benzodiazepines or other hypnotics,[72,73] and with the combination of ketamine, methomidate, and azaperone there were no adverse effects seen in *paper III*.[74] These anaesthetic agents were therefore chosen in *paper IV*. The use of ketamine before clamping and unclamping of LAD was used to reduce pain and stress and thereby maybe also the risk of ventricular fibrillation.

Hamburger et al [52] reported that the common problem with ventricular arrhythmias requiring administration of a variety of antiarrhythmic drugs and/or extensive defibrillation during the occlusion or at reperfusion [75-78] could be reduced to ~20% by ultra-short acting thiobarbiturate anaesthetic, Thiamylal®.[52]
The chosen anaesthetic technique in *paper IV* resulted in a similar low frequency of severe arrhythmias of about 20% (18%) as reported by Hamburger et al[52] indicating that the anaesthetic procedure was adequate for this experimental model.

Treatment Fluids *(I, II)*

On the basis of the pathophysiological response of SHR to haemorrhage our hypothesis was that a glucose-osmotic support would be more beneficial for reversal of hyopvolaemia in primary hypertension than sodium ionic hypertonicity. Therefore the fluids chosen for the resuscitation treatment in *paper I* were hypertonic glucose (HG) and hypertonic saline (HS). A third fluid, normal (isotonic) saline, was used for testing if normal saline with equal sodium load as in HS is more beneficial than hyperosmolarity per se.

In the present study the usually recommended small-volume resuscitation of 4 ml/kg b.w was not given of HG and HS but instead 4.5 ml/kg of b.w. This means that a slightly larger percentage of the shed blood volume was replaced than in other studies of hypertonic treatment of haemorrhage. This larger volume replacement was necessary due to the known poor tolerance of SHR to blood loss.[57,58,60]

Since the effects of HS on ischaemia-induced cardiac dysfunction in resuscitation of shock are still not known in detail, HS was used in *paper II*. 3% of HS was the required concentration to increase Na^+ in the perfusate to ~150- and ~160 mmol/l. In retrogradely perfused, nonworking, isolated rat hearts, HS has been reported to depress myocardial function by influencing contractility and relaxation rate.[44] The importance of the sodium ion as compared to osmolality *per se* for the efficacy of hypertonic fluid therapy in shock has been documented. A high plasma sodium level seems essential for survival.[31,32]

In the present study a two-step increase in the sodium concentration of the perfusate was carried out, allowing us to assess the effects of increasing sodium level on cardiac performance. Perfusate sodium levels of ~150 mmol/l were reached after the first and ~160 mmol/l after the second addition of HS, i.e. sodium levels similar to those achieved when HS is used in experimental[36,79] or clinical shock treatment.[80,81] Therefore the ionic and osmolar changes reached at the myocardial cell level in the present study should be representative for *in vivo* situations.

Treatment Fluids *(III, IV)*

At reperfusion after hypovolaemia-shock induced tissue ischaemia caused by haemorrhage, sepsis, burns, surgery, and in revascularization of coronary arteries and other vessels, there is of importance to choose the therapy inclusively the resuscitation fluids with the thought to diminish the ischaemia-reperfusion injury. Therefore, HS with or without dextran were used in *paper III* (acute limb ischaemia-reperfusion) and *paper IV* (acute myocardial ischaemia-reperfusion) as therapy fluids because of their properties. Dextran 70 was included as oncotic component due to its specific effects on the rheological behaviour of blood.[82,83] Furthermore, dextrans exert pharmacological effects on red cell aggregation, platelet function, plasma viscosity, and leukocyte-endothelial cell interactions.[84-87]

Therefore, the combined effects of dextran, including its potential as a free radical scavenger, and HS, with its oedema reducing capacity,[88] were considered of potential value for reduction of both limb tissue, and myocardial damage in the experimental models Dextran 70 has been suggested to be the colloid of choice to combine with HS in the treatment of shock and ischaemia.[16,19,21,34,35,89] Studies comparing dextran and hydroxyethylstarch (HES) suggest that dextran is preferable due to its specific beneficial microcirculatory effects by which ischaemia-induced leukocyte-endothelial cell interaction is attenuated.[90] Dextran 70 has also been suggested to exert specific beneficial effects at reperfusion.[35,44,91] In addition to enhanced free radical scavenging[44] dextran will further enhance microcirculation by the attenuation of leucocyte-endothelial interactions.[35,91]

The Role of the Infusion Rate

It is usually considered that a rapid infusion of hypertonic solution is mandatory for an efficient mobilization of endogenous fluid.[29] However, rapid administration of HS has been suggested to decrease the vascular perfusion pressure. An immediate and severe hypotension before cardiovascular improvement secondary to a decrease in systemic vascular resistance was seen after rapid administration of HS solution (HS 7.5%, 3 ml/kg infused intravenenously for 1 min).[43] In these studies the infusion was very fast and Kien et al[43] conclude that acute hypotension caused by rapid infusion of HS was not mediated by cardiac depression but by a decrease in total peripheral resistance. This was reported already in 1961 as a cholinergic-like effects of hypertonic solutions infused for 10 secs in amounts of 1 ml/kg b.w.[8] (HS 5-8%, hypertonic glucose 50%, urea 12,5-50%).

In a shock study of intestinal ischaemia infusion of HS (1.7 and 10 ml/kg/min) resulted in an immediate severe blood pressure decrease associated with cardiac arrythmias and/or breathing difficulties.[42] In the SHR study (*paper I*) no hypotensive effect of HS was seen with a slow infusion rate (0.45 ml/kg/min) and arrythmias were only observed premortally similar to the other groups. An efficient initial increase in MAP and maintenance of increased serum sodium at 30 and 90 min after treatment indicated a good volume supporting effect of the infusion rate used. Also in study *III*, and *IV* the treatment fluids were given very slowly at a rate of 0.4 ml/kg/min. The increased serum sodium and osmolality, simultaneously with dilutional effects shown as reduction of serum potassium, and proteins as well as the haemodynamic response indicate that the infusion rate was effective. In *paper II* the HS of 3% was thrown into and mixed with the perfusate in a reservoir and in part into the 130 cm long chamber to reach ~150- and ~160 mmol/l Na^+.

Measurements of Central Haemodynamics, Muscle Metabolites, and Skin Blood Flow *(III)*

Transthoracic Electrical Bioimpedans (TEB)

TEB was used in *paper III* for the cardiac output determination since Jivegård et al[54] documented a close correlation in the pig between electrical bioimpedance and thermodilution measurements under varying experimental conditions.

Muscle Metabolites

The ATP, CrP, and lactate levels of skeletal muscle were used for the assessment of the metabolic alterations induced by the incomplete limb ischaemia. We have previously demonstrated significant relationships between alterations of these metabolite levels and the membrane function (transmembrane potential) of skeletal muscle cells during conditions of shock and ischaemia.[92]

Laser Doppler Flowmetry - LDF

The laser Doppler flowmetry technique used for continuous monitoring of microvascular perfusion within tissues was described by Nilsson et al.[93] Laser Doppler flow measurements provide a noninvasive evaluation of local skin blood flow and has previously been used in porcine and man studies,[51,53] and the method was considered to be suitable for continuous measurements of skin blood flow in this study.

Measurements of Area at Risk and Infarct Size *(IV)*

TTC-Staining

The TTC staining technique has previously been used in other studies and shown to adequately differentiate viable from non-viable myocardium in the area at risk after LAD occlusion.[52,75,76,94] It has been pointed out that the critical variable for the occurrence of significant myocardial damage is the period of coronary artery occlusion, and a 15 min period has been considered insufficient for infarction.[75] The break point between significant and non-significant damage has been suggested to be at ~45 min or longer.[76] The 45 mins duration of LAD occlusion chosen in the present study demonstrated a significant myocardial damage.

Planimetrical Measurement

A planimetrical method using the processing and analysis program, NIH Image 1,54 for the Macintosh has previously been used for measurements of infarcted areas and areas at risk.[52] The method was used in our study and two individuals measured the areas independently. The correlation between the two observers showed coefficients of correlation for the measured areas of 0.91 for Area at risk, 0.97 for infarcted size of Area at risk, and 0.94 for infarct size of left ventricle. The mean values of the two observers measurements were used in the study. Theese procedures were considered adequate for measurement of the different areas.

6. General Discussion

This thesis is based on four experimental studies in rats and pigs with focus on hypertonic-hyperoncotic volume loading with hypertonic glucose and hypertonic saline with or without dextran 70 during a variety of conditions with impaired cardiac performance, depressed liver function and cardiovascular reserve.

Although osmolality has been considered important in the etiology of shock,[10,95] the essential role of sodium has also been documented.[31,32] Experimental studies have demonstrated the importance of hyperosmotic resuscitation resulting from fluid mobilization to the intravascular compartment in particular after hypovolaemia due to haemorrhage.[29] HS has been reported to increase cardiac output[6,14,25,36,96,79] as a result of a combination of increased cardiac contractility, plasma volume expansion, and peripheral vasodilation.[97] However, these findings were demonstrated in subjects with normal cardiac function, studied in intact animals[96] and hypovolaemic shock.[6,14,25,36,79,97] Questions have been arisen whether subjects with compromised cardiac function would react in a similar way, or be deleterious[6] because of an increased oxygen consumption when HS exerts a positive chronotropic effect.

Improvement of microvascular blood flow is necessary for reversal of the microcirculatory dysfunction caused by ischaemia and reperfusion.[35] Hypertonic solutions may salvage the micro-circulation both by deswelling endothelial cells and corpuscular blood elements, and by inhibiting leucocyte adhesion to endothelial surfaces.[29,98] Such effects are of importance for the integrity of the endothelial barrier function and thereby for the transfer of fluid from the vessels into the interstitial space in the reperfusion period.[29,98]

Menger et al[99] investigated microvascular injury induced by ischaemia-reperfusion in striated muscle and they proposed that within the microvascular network three components contribute to ischaemia-reperfusion (I/R) -induced reflow paradox, i.e., generation of oxygen radicals, release of chemoattractants, and adherence of leucocytes, which may induce lipid peroxidation, membrane disintegration, and loss of endothelial integrity, finally resulting in increased microvascular permeability, cell death, and tissue damage.[99]

After prolonged periods of ischaemia under nonisovolumetric conditions a patchy reperfusion occur when perfusion buffer is reintroduced to the coronary circulation. This is called the no-reflow phenomenon, and is due to capillary "shut down" and occurs not because of thrombosis but rather because osmotic swelling of the cardiac myocytes or tissue oedema that constricts the capillaries.[100,101]

Hypertonic Fluids Administration after Haemorrhage in the SHR

(paper I)

A deficient glucose mobilization of SHR following haemorrhage has previously been reported[60] and in accordance maximum glucose levels reached only ~8 -10 mmol/l in paper I, as compared to ~20 mmol/l in normotensive rats.[60] The hyperglycaemic response was also of short duration in the present study with normo- to hypoglycaemic levels reached at 30 mins after haemorrhage in untreated control rats.

Since the hyperglycaemic response to blood loss seems attenuated in SHR,[60] HG was expected to be of particular benefit. With the small-volume HG regimen, hyperglycaemia was prolonged in the present study, but still the haemodilutional effect of HG was less pronounced than that of HS. The greater volume-expanding effect from HS as compared to HG might be explained by a distribution of sodium ions mainly within the extracellular space, while glucose is also taken up and metabolized by tissue cells. The more pronounced haemodilution after HS than after HG treatment is considered to have contributed to increased venous return thereby increasing cardiac output and regional blood flow.[34,102-104]

In particular splanchnic perfusion has been shown to improve in response to HS.[97] This should be of specific value in the treatment of blood loss since liver perfusion is known to be critically reduced by hypotension[105] and liver metabolic disturbances induced by haemorrhagic hypotension seem even more critical in hypertensive disease.[57,58]. The increased hepatic vulnerability in hypertensive disease is probably not due to disturbances in cellular function[59] but rather to the presence of structural changes in hepatic and portal vessels.[106,107] Reduced blood flow to the liver and thereby inadequate glycogenolysis and gluconeogenesis must, therefore, be the main explanation for the impaired glucose mobilization of SHR in response to haemorrhage and for the poor shock tolerance of SHR.[57,60] It is surprising that the rather massive load of glucose did not induce any pronounced hyperglycaemia. The explanation could be that pancreas is still intact with capacity to produce insulin according to of the increased blood glucose.

The resuscitation with HG, thus, did not correct the disturbed glucose metabolism, but rather increased lactate production. Lactate levels, thus, increased more after HG than after HS or NS, and were of similar magnitude in this group as in untreated bled SHR. The present results therefore indicate a poorer improvement of the splanchnic perfusion in response to HG than to HS or NS treatment.

Hyperosmolar solutions have been claimed to exert beneficial effects on cardiac performance.[41,96,97] In the treatment of shock in SHR, this may be of specific value since SHR seem to have a poorer cardiac performance than normotensive rats at low perfusion pressures.[108] In addition, already in the basal state SHR have an increased sympathetic activity[109,110] which together with venous structural changes[107] could reduce the whole-body venous capacity.[111-113] The remaining capacity of the venous system to maintain an adequate cardiac filling pressure after blood loss therefore seems limited.[114] Reduced venous return and impaired cardiac performance thus seem to be critical factors for SHR after blood loss. Hypertonic glucose could then be expected to be beneficial, since it has been shown to improve ventricular function in nonischaemic as well as postischaemic hearts in normotensive rats while HS alone did not improve myocardial function.[44]

Both HG and HS were found to exert positive chronotropic effects. The cardiostimulatory effect seemed more pronounced after HS than HG, since increase in arterial pressure was higher after HS. The higher MAP level after HS could be due to better transcapillary refill compared with HG which might in turn have improved cardiac performance. However, the MAP increase was more pronounced immediately after start of treatment, indicating a possible direct cardiostimulatory effect or more generalized effects of an enhanced sympathetic activity by HS.

Cerebroventricular administration of HS is known to enhance sympathetic activity.[115] The cardiovascular changes observed after intravenous small-volume resuscitation with 2400 mOsm/l HS during severe haemorrhagic shock[6,14] are similar to those induced by intraventricular infusion of HS.[116] Increased sodium levels of ~15-20 mmol/l in the cerebrospinal fluid, which are levels comparable to those reached in plasma after standard HS treatment, have been shown to improve cardiovascular function and increase the tolerance to hemorrhage.[116] The observed beneficial effects of HS on cardiovascular performance after hemorrhage in the present study could therefore be mainly due to activation of cerebral sodium sensors rather than to direct cardiostimulatory effects.

NS seemed as effective as HS for resuscitation of haemorrhage in SHR. The major disadvantage of NS resuscitation is the large volume requirements which include a risk of oedema formation. Oedema generally appears after haemorrhagic shock, but different opinions have been presented concerning the distribution of extravascular fluid. Calculations of the cellular fluid content based on membrane potential measurements[117] and direct microvascular observations[35] suggest that circulatory shock rapidly causes cellular oedema in heart, muscle and endothelial cells. On the other hand, distribution of tracers suggests that an increase in intracellular water content does not always occur after shock, but interstitial expansion will usually follow fluid resuscitation therapy.[118] The hypovolaemia reversing effects obtained with NS in *paper I* were comparable to or even better than those of HS in spite of the fact that a volume of only 2.1 times the shed blood volume was infused. The fact that both treatments included the same amount of sodium ions and were comparable in efficiency for resuscitation point to the importance of sodium for maintenance of blood volume and extracellular fluid.

The good survival of NS treated SHR emphasizes the importance of adequate volume loading for successful treatment of acute haemorrhage. The hypertonic fluid regimens used in the present study should consequently either include colloid or be followed by additional fluid infusion to achieve optimal posthaemorrhagic survival because the effect of small-volume hypertonic solutions is short-lived.[119]

HS and Ischaemia-Reperfusion in Vitro
(paper II)

In the study of isolated, antegradely perfused, working rat heart no direct positive inotropic effect of hypertonic saline on myocardial function could be demonstrated. Both additions of HS to the nonischaemic as well as to the ischaemic isolated heart caused consistent, transient depressions of cardiac performance, as shown by reduction of stroke volume lasting for ~3 mins. Since the hearts were paced at a constant heart rate and kept on constant preload and afterload, the observed reduction in stroke volume is due to depressed contractility. Similar depression of ventricular function by HS in a nonischaemic and postischaemic isolated, retrogradely perfused nonworking Langendorff heart preparation has been reported by Brown et al.[44] The present study indicates that HS may enhance the functional detoriation of the isolated heart preparation, since nonischaemic as well as ischaemic hearts exposed to HS were found to maintain a poor stroke volume over time when compared with control hearts exposed to ischaemia only *(Figure 9)*.

Shock resuscitation with hyperosmolar solutions is usually reported to be beneficial due to improved cardiac output and tissue perfusion.[6,14,25,36,79,96] Results from studies[6,14,40,41] of intact animals have suggested that myocardial contractility may be increased by hypertonic saline. In isolated cardiac tissue a negative inotropic effect of increased sodium concentration was previously reported by Bassini et al[120] and Tillisch and Langer.[121] Part of the myocardial depressant effects of hypertonic saline may be due to loss of water from the muscle fibers.[122] Thus, extracellular as well as intracellular sodium concentrations are increased i.e., changes in concentration gradient are induced that are associated with decreased myocardial contractility.[123] An increased ratio of the internal/external sodium activities may lead to a decrease in intracellular calcium activity via sodium-calcium exchange and thereby a negative inotropic effects.[123]

The myocardial metabolic effects of HS administration were only seen in the nonischaemic heart and as transiently reduced myocardial oxygen consumption. A concomitant decrease in coronary flow caused by autoregulation maintained the myocardial oxygen extraction at a constant rate.

The observations of a myocardial-depressant effect of HS on the isolated nonischaemic and ischaemic working rat heart in *paper II* favour the concept that the reported stimulatory effects of HS on myocardial performance in shock resuscitation must be mainly indirect through central mechanisms. Infusion of hypertonic saline has been shown transiently to increase plasma catecholamine levels.[124] Liang and Hood[40] considered this result to be at least in part responsible for the inotropic action of hypertonic saline. The observation of increases in intracerebroventricular or intracerebral sodium concentration similar to those in blood after hypertonic saline resuscitation[116] could indicate that activation of central cerebral mechanisms may be important. Activation of a pulmonary reflex was originally suggested by Rocha-e-Silva et al,[104] although the contribution of such a reflex to the beneficial haemodynamic effects of hypertonic saline in shock resuscitation has been questioned.[125] Attenuation of the plasma activity of a myocardial-depressant factor has been another suggested beneficial effect of hypertonic saline.[37] Since no direct positive inotropic effect could be demonstrated from HS in isolated hearts, the cardiostimulatory effect and efficiency of HS resuscitation seems to be mainly due to central sympathetic activation.

Hypertonic-Hyperoncotic Fluids Administration in Lower Limb Ischaemia-Reperfusion in the Pig
(paper III)

In vascular surgery it is well known that acute non-traumatic lower limb ischaemia is associated with high mortality rates, often exceeding 25%.[126] It is possible that the high mortality rates in connection with acute limb ischaemia could be reduced if the surgical treatment is combined with intravascular fluid regimens that more rapidly and efficiently improve tissue perfusion by enhancing microvascular blood flow, reducing blood cell-endothelial cell interactions, and diminishing tissue oedema formation following reflow.

It has previously been shown that the aortic occlusion reduces the blood flow in the femoral vein from about 125 ml/min to 4 ml/min reducing correspondingly tissue oxygen tension.[51] The demonstrated increase in skeletal muscle lactate content and reduction of high energy phosphagens during the 4 h period of incomplete ischaemia in *paper III* therefore clearly indicate reduced regional tissue oxygen availability and partly anaerobic cellular metabolism.[92,127,128] Systemic derangements of the acid-base balance during the period of incomplete limb ischaemia were not observed indicating that the systemic effects of the local hypoxic events were centrally compensated.

During infusion of the different fluids prior to the deflation of the aortic balloon a more efficient blood volume expanding effect was obtained with HS and HSD than with NS, as shown by the changes in CVP, MAP and TFI. TFI was in this study assumed mainly to reflect the central venous return. Reflow following complete as well as incomplete limb ischaemia is consistently accompanied by hypotension as observed in the present study. Following reflow this initial central haemodynamic deterioration was less pronounced in the HS and HSD groups than in the NS group. It has previously been documented that the infusion of a hypertonic solution results in a dynamic fluid redistribution from extravascular sources into the vascular compartment.[29] Such a fluid mobilization may not be expected at the infusion of 4 ml/kg of NS which clearly is a suboptimal fluid volume.

Also the volume expanding effect of HS *per se* is transient and of relatively short duration due to a redistribution back into the extravascular sources.[29] Therefore the use of HS in combination with a colloid is usually advocated since haemodynamic stability thereby will be maintained for a more prolonged period of time.[21,16] In the present study MAP was also better maintained over time in the post-ischaemic period in the HSD than in the HS and NS groups.

The microcirculation through the capillaries has been reported to be restituted more efficiently by hyperosmotic-hyperoncotic solutions[34,35] resulting in improved tissue nutrition.The somewhat better clearance of lactate from skeletal muscle and more rapid restitution of the high energy phosphagen levels in the HSD group favour such a concept.

The role of leukocytes in the pathophysiology of skeletal muscle ischaemic injury has been well documented[99,129] and leukocyte depletion has been shown to attenuate post-ischaemic vascular injury.[130] Dextran may not only modulate the endothelial surface charge of capillaries but also reduce endothelial permeability.[86] A dextran related reduced access of different molecules to the cell surface by steric hindrance and/or electrostatic shielding of the glycolyx may explain such effects.[86] Consequently dextran seems to modulate several of the pathopysiological events that occur in the post-ischaemic reflow period, i.e. events related to "no-reflow" phenomena (white blood cell plugging of capillaries), release of inflammatory mediators, and formation of oxygen-derived free radicals. Such beneficial effects of dextran in combination with hypertonic saline on microvascular blood flow in the post-ischaemic period are indicated in the present study by the more efficient metabolic restitution of the ischaemia-induced metabolic disturbances in skeletal muscle in the HSD group.

Tissue oedema formation at reflow in the post-ischaemic period is considered a factor influencing microvascular blood flow and tissue oxygen supply. It has recently been shown that hypertonic-hyperoncotic fluid treatment will efficiently mobilize oedema induced by hydrostatic factors or inflammatory mediators such as bradykinin.[88] Vascular ischaemia, as seen in connection with peripheral vascular or major aortic vascular surgery is often associated with post-ischaemic fluid retention and oedema formation. In connection with aortic surgery quite often major body weight changes of up to 7-8% have been noted.[131] By the use of hypertonic intravenous fluids during aortic surgery it has been shown that haemodynamic stability can be well maintained at less volume transfusion.[131,132] Thereby the risk of fluid overload and extensive increase in body weight may be prevented. Intraoperative combined use of hyperosmotic/hyperoncotic solutions may be even more advantageous to avoidance of tissue fluid accumulation.[133] Our observations of enhanced post-ischaemic metabolic recovery in the 7,5% HS 6% dextran 70 group indicate that even a low colloid content seems to enhance the post-ischaemic tissue metabolic recovery but a higher colloid concentration could be even more effective.[119]

Hypertonic-Hyperoncotic Fluids Administration in Myocardial Infarction-Reperfusion in the Pig
(paper IV)

Experimental and clinical studies in severe haemorrhagic shock have shown beneficial effects of small volume of HS ~7.5% with or without colloid on cardiovascular performance.[6,14,19,21,25,31,36,37,39]

In trauma victims with already impaired left ventricular function there is a high risk for development of either transient cardiac ischaemia or acute myocardial infarction due to hypotension after major haemorrhage and causing hypoperfusion of the myocardium. The value of resuscitation fluid is important and may prevent myocardial ischaemia. In patients with right ventricular infarction the preload increase after fluid loading is important as shown in cardiogenic shock due to right ventricular infarction[80] whereas in patients with left ventricular infarction volume loading can cause left ventricular failure. The effects of HS/HSD therapy of shock in patients with depressed left ventricular function have not been investigated so far.

The effects of hypertonic solutions on the left ventricle performance have been studied in cardiac tissues samples, in isolated heart preparations, and in different experimental and clinical settings but the functional role of such treatment has still not been conclusively clarified.[10,40,41,43-45,80,97,120,134-137] When HS is administered in the treatment of haemorrhagic shock the left ventricle is usually not compromised due to myocardial injury and the left ventricle is capable to react normally if preload can be increased. This may explain the reported beneficial cardiovascular effects of HS or HSD in the treatment of haemorrhagic hypotension.[6,14,39]

In paper IV the effects of hypertonic saline±dextran 70 in left ventricular myocardial infarction and reperfusion were investigated. The occlusion of LAD during a 45 min period and thereafter a 240 min period of reperfusion allowed us to study the animals during a longer period and recording haemodynamic and metabolic effects of HS and HSD and development of the myocardial insult after induced left ventricular ischaemia and reperfusion.

The infusion of HS and HSD resulted in expected increases of serum Na^+ and osmolality. The haemodilutional blood volume expansion was evidenced by reduced K^+, Ca^{++}, plasma protein levels, and haematocrit readings. The fluid mobilization induced by HS and HSD was furthermore reflected by increased right and left ventricular filling pressures. The positive haemodynamic effects initially induced by HS and HSD were mainly seen as increased CI at the end of the infusion but the changes were of short duration, especially for HS. The combination of HS and dextran increased cardiac performance as opposed to HS alone due to the colloid osmotic effects and prolonged intravascular persistence of dextran 70.[83]

Volume loading by HS or HSD infusion may further increase an already increased preload of the failing ischaemic left ventricle. This could explain that myocardial contractility (+dP/dt) was not found to be enhanced but instead significantly decreased in the HS treated animals at 10 mins after the end of the infusion. However, even if CVP and PCWP were enhanced by HS and HSD administration in the present study, they did not reach that levels seen in heart failure. When plotting Frank-Starling curves (left ventricular stroke work index (LVSWI) versus left ventricular end-diastolic pressure (LVEDP) HS treatment was found to cause a prolonged functional depression, while functional depression after HSD treatment was not obvious until towards the end of the reperfusion period. Such differences could reflect the beneficial influences of dextran, as a free radical scavenger and promotor of capillary blood flow, on myocardial performance in the post-ischaemic period. This would be in agreement with the suggestion of Brown et al[44] that the post-shock benefit of HSD is unrelated to direct myocardial effects of saline but rather to toxic oxygen metabolite scavenging by dextran.

In the treatment of cardiogenic shock due to right ventricular infarction[80] volume expansion will increase preload in the right ventricle necessary for the failing ventricle to produce an adequate stroke volume. In such a situation volume loading with hypertonic solution is important for normalizing left ventricular preload. When, on the other hand as in the present study, the left ventricle is damaged by an infarct the situation is quite different.

The initial change of heart rate (HR) was found to be small in all groups at the reperfusion in the present study. This is in contrast to the findings of other studies reporting increased HR in response to treatment with HS.[41] The lack of major HR changes in response to treatment could reflect an already high sympathetic tone caused by the acute myocardial infarction[138] or an high vagal tone.

Since HR did not differ among the three experimental groups, HR could probably not be a determinant parameter explaining the difference in contractility state between the groups.

In *paper II* we found that repeated additions of HS to nonischaemic as well as to ischaemic, isolated, anterogradely perfused, working rat hearts caused transient depressions of cardiac performance following each HS administration.[45] Since HR was paced at a constant rate and at unchanged left atrial pressure in that experimental model the observed reduction in stroke volume was assumed mainly to reflect direct negative inotropic myocardial effects. Depressant rather than stimulatory effects of HS on myocardial performance has been indicated also in other experimental studies.[44,135] The cardio-depressant effect has been claimed to be due to large HS doses or very fast infusion rates.[4,43] In the present study the commonly used dose of 4 ml/kg b.w. was infused over a period of 10 min which is supposed to be a slow infusion. In a recently published study of HS infusion by Kien et al,[139] the contractile function and blood flow were investigated in regions of normal myocardium and ischaemic myocardium after occlusion of LAD. The authors found that contractile function and blood flow were improved in the normal myocardium after HS infusion but decreased in the region distal to coronary occlusion (ischaemic myocardium) and that this could lead to worsening of ischaemic injury. They concluded that HS may be deleterious in hearts with impaired contractile function caused by ischaemia and also that additional studies are warranted to investigate the safety of HS in patients with impaired myocardial function due to coronary vascular disease.

In our study the measured area of infarct size and area at risk did not differ between the groups and thereby was not the ischaemic injury worsened by HS and HSD compared to normal saline infusion at reperfusion after LAD occlusion *(Figure 14)*. Although HS/HSD has previously been suggested to exert beneficial effects on outcome when used for resuscitation of shock and hypovolemia[14,19,21,25,29,31,36,37,39] no reduction of the myocardial ischaemic insult could be demonstrated in the present study. There were no differences seen between the groups regarding myocardial metabolites at the end of the reperfusion period *(Figure 13)*. The release of serum markers of myocardial damage did not differ with the exception of ASAT which in the reperfusion period was significantly more increased in the HSD group compared to NS and HS. This could possibly reflect a better washout from the ischaemic area resultant from the positive microcirculatory effects of dextran.

Potential Clinical Use of Hypertonic-Hyperoncotic Therapy with HSD in Patients with Depressed or Infarcted Left Ventricle.

In elderly patients undergoing surgical procedures coronary artery disease is often present also in the asymptomatic patients and asymptomatic left ventricular dysfunction may also be present. In case of major haemorrhage causing hypovolaemia these patients are at risk developing myocardial infarction peroperatively.

Hypertonic saline-6% hydroxyethyl starch has been used effectively for preoperatve hemodilution in cardiac surgery in patients with coronary artery disease,[139] and is also found effective for postoperative fluid replacement in patients after cardiac revascularization.[140] Administration of HS with or without colloid as fluid replacement during reconstructive vascular surgery has not been investigated and consequently a need to clarify if the use of these fluids could worsen an ischaemic and postischaemic stunning.

In patients undergoing revascularization with percutaneous transluminal coronary angioplasty (PTCA) after acute myocardial infarction the use of high doses of thrombolytic agents, for example heparin, could cause major haemorrhages. Instead of, or in combination with a more moderate dosage of the used thrombolytic agent, administration of hypertonic saline-dextran 70 may be of value, due to its oedema reducing capacity[88] and positive rheological effects[82,83] on red cell aggregation, platelet function, plasma viscosity, and leucocyte-endothelial cell interactions.[84-87,91] Dextran 70 has also been suggested to exert specific beneficial effects at reperfusion,[35,44,91] and in addition to enhance free radical scavenging,[44] which is a merit since it is known that oxygen free radicals can increase the expression of leucocyte adhesion molecules on endothelial wall.[141,142]

7. Conclusions

I Hypertonic (2400 mOsm/l) glucose was less favourable than hypertonic saline for resuscitation after blood loss in the spontaneously hypertensive rat, due to less pronounced intravascular volume expanding and arterial pressure supporting effects resulting in increased lactate load. In this experimental model the sodium load therefore seemed more beneficial than the hyperosmolarity per se. However, adequate restitution of the lost fluid volume seems even more important for survival since about twice the bled volume given as isotonic 0.9% saline was found to be more effective than the same sodium load given as 7.5% NaCl solution.

II The present results on an isolated paced, working rat heart preparation at controlled preload and afterload show that hypertonic saline exerts rapid transient as well as more prolonged myocardial depressive effects both under ischaemic and nonischaemic conditions.
These observations indicate that the beneficial haemodynamic effects of hypertonic saline in shock treatment must be mediated mainly via systemic rather than direct myocardial mechanisms.

III On the basis of the present study it is concluded that the infusion of small volumes (4 ml/kg of body weight) of hypertonic saline 6% dextran 70 at the time of tissue reperfusion following prolonged subtotal tissue ischaemia constitutes an efficient fluid regimen for reversal of ischaemia-induced haemodynamic and tissue metabolic disturbances.

IV The infusion of hypertonic saline at reperfusion after 45 mins of myocardial ischaemia caused by LAD occlusion did not effectively improve haemodynamics or myocardial contractility and did not reduce or increase the myocardial damage in the post-ischaemic period. The combination of hypertonic saline and dextran 70 seemed to be more effective for improving the haemodynamics and myocardial contractility, but neither reduction or increase of infarct size or area at risk could be demonstrated.
Therefore, the present data do not clearly demonstrate that hypertonic saline treatment is of value for reduction of ischaemia induced acute myocardial damage.

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10. Original Papers

I Efficacy of osmolality and ionic composition of resuscitation fluids for treatment of acute blood loss in the spontaneously hypertensive rat (SHR).

II Effects of hypertonic saline on myocardial function and metabolism in nonischemic and ischemic isolated working rat hearts.

III Hypertonic saline infusion with or without dextran 70 in the reperfusion phase of experimental acute limb ischaemia.

IV Hypertonic saline (HS) ± dextran 70 administration in acute myocardial infarction and reperfusion.

På grund av upphovsrättsliga skäl kan vissa ingående delarbeten ej publiceras här.
För en fullständig lista av ingående delarbeten, se avhandlingens början.

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