Is oxygen supply a limiting factor for survival during rewarming from profound hypothermia?

Timofei V. Kondratiev,¹,² Kristina Flemming,¹,² Eivind S. P. Myhre,³ Mikhail A. Sovershaev, and Torkjel Tveita¹,²,⁵

¹Department of Anesthesiology, Institute of Clinical Medicine, Departments of ²Medical Physiology and ³Biochemistry, Institute of Medical Biology, University of Tromsø, ⁴Department of Anesthesiology, University Hospital of North Norway, Tromsø; and ⁵Section of Cardiology, Department of Medicine, Sørlandet Hospital, Kristiansand, Norway

Submitted 21 November 2005; accepted in final form 30 January 2006

Kondratiev, Timofei V., Kristina Flemming, Eivind S. P. Myhre, Mikhail A. Sovershaev, and Torkjel Tveita. Is oxygen supply a limiting factor for survival during rewarming from profound hypothermia? Am J Physiol Heart Circ Physiol 291: H441–H450, 2006. First published February 3, 2006; doi:10.1152/ajpheart.01229.2005.—It has been postulated that unsuccessful resuscitation of victims of accidental hypothermia is caused by insufficient tissue oxygenation. The aim of this study was to test whether inadequate O₂ supply and/or malfunctioning O₂ extraction occur during rewarming from deep/profound hypothermia of different duration. Three groups of rats (n = 7 each) were used: group 1 served as normothermic control for 5 h; groups 2 and 3 were core cooled to 15°C, kept at 15°C for 1 and 5 h, respectively, and then rewarmed. In both hypothermic groups, cardiac output (CO) decreased spontaneously by >50% in response to cooling. O₂ consumption fell to less than one-third during cooling but recovered completely in both groups during rewarming. During hypothermia, circulating blood volume in both groups was reduced to approximately one-third of baseline, indicating that some vascular beds were critically perfused during hypothermia. CO recovered completely in animals rewarmed after 1 h (group 2) but recovered to only 60% in those rewarmed after 5 h (group 3), whereas blood volume increased to approximately three-fifths of baseline in both groups. Metabolic acidosis was observed only after 5 h of hypothermia (15°C). A significant increase in myocardial tissue heat shock protein 70 after rewarming in group 3, but not in group 2, indicates an association with the duration of hypothermia. Thus mechanisms facilitating O₂ extraction function well during deep/profound hypothermia, and, despite low CO, O₂ supply was not a limiting factor for survival in the present experiments.

resuscitation; oxygen transport; oxygen consumption; heat shock protein 70; blood volume

Little is known about the pathophysiology of tissue oxygenation during hypothermia in homeothermic experimental animals. Deranged tissue oxygenation has been postulated to be an important determinant for survival during cooling (several hours) hypothermia as well as during rewarming after acute (a few hours) hypothermia. Hypothermia decreases the metabolic rate of a homeothermic organism. In clinical medicine, deliberate hypothermia has been used to depress metabolic rate and, thus, vulnerability of the tissue to ischemic damage. Cooling slows tissue body O₂ consumption (VO₂) and CO₂ production in parallel: ~4–9% per 1°C (6, 14, 27).

Short exposure time and low level of hypothermia are accepted as essential factors for a favorable outcome after rewarming. Thus, when hypothermia is used during, e.g., cardiac surgery, time and degree of temperature reduction are kept to a minimum. On the contrary, a victim of accidental hypothermia is frequently exposed to deep hypothermia for hours. This prolonged exposure and deep hypothermia are strongly related to failure of the victim to survive during rewarming. Striking similarities have been found between unsuccessful rewarming of human patients from accidental hypothermia and unsuccessful reanimation of animals after exposure to acute experimental hypothermia (7, 32). The challenge when patients are rewarmed from accidental hypothermia is circulatory dysfunction, which may lead to circulatory shock, also termed “rewarming shock,” i.e., a decline in cardiac output (CO) and a sudden drop in blood pressure. In some studies, the mortality for patients entering a rewarming shock is as high as 70% (26).

For obvious reasons, it is necessary to use experimental animal models to elucidate pathophysiology related to accidental hypothermia. However, conflicting results have been reported from such experiments. With respect to reanimation, experiments using intact dogs report that rewarming after acute (7), as well as prolonged (32), mild (32–35°C) hypothermia (41) might be unsuccessful. Failure to survive has been suggested as a result of inadequate tissue perfusion due to changes in the peripheral vascular bed and/or myocardial function. Other researchers have reported that O₂ supply seems to be adequate during hypothermia followed by restitution of the circulatory function after rewarming (24). In contrast, reduced cardiac function after rewarming, unrelated to limited myocardial O₂ supply or VO₂ was found in dogs after 5 h of exposure to mild and moderate hypothermia (36). From other experiments on dogs, tissue VO₂ was maintained during cooling to 29°C (12). Few reports have investigated effects of exposure to deep (20–25°C) or profound (14–19°C) hypothermia and rewarming. In 1965, Popovic and Kent (28) showed that rats cooled to a body temperature of 15°C lived for 9–10 h. However, the animals did not survive rewarming if hypothermia lasted >5 h. It was suggested that the cause of death in hypothermia was due to a limited O₂ supply due to progressive temperature-dependent circulatory failure. Furthermore, it was proposed that similar mechanisms could cause death in these animals during early stages of rewarming (28°C).

Therefore, it is possible that circulatory instability during rewarming not only results from deterioration of心血管...
lar function, but also from reduced O$_2$ supply, as well as inability to increase O$_2$ extraction to compensate for low CO during deep hypothermia. The aim of the present study was to investigate these aspects in our specially designed intact animal model. The specific aims were as follows: 1) to investigate possible differences in hemodynamic function and O$_2$ supply in response to 1 h vs. 5 h of hypothermic exposure, 2) to explore whether changes in determinants of O$_2$ transport, such as blood volume, hematocrit (Hct), hemoglobin (Hb), or ability to increase O$_2$ extraction, depend on the duration of hypothermia, and 3) to measure protein markers of myocardial tissue injury or tissue stress, e.g., creatine kinase muscle-brain isoform (CK-MB) and heat shock protein 70 (HSP70).

**MATERIALS AND METHODS**

Male Wistar rats (280 – 400 g) classified for microbiological status according to recommendations of the Federation of European Laboratory Animal Science Associations were provided by Harlan. On arrival, the animals were quarantined for 1 wk. During the experiments, the animals were housed in accordance with guidelines for accommodation and care of animals outlined in Article 5 of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18.III.1986). The animals were allowed free access to food and water. The experimental protocol was approved by the Norwegian Animal Research Authority and conducted accordingly.

**Anesthesia**

Anesthesia was induced by infusion of pentobarbital sodium at 50 mg/kg body wt ip followed by continuous infusion at 7.5 mg·kg$^{-1}$·h$^{-1}$ through an intravenous line in the right jugular vein extended to the right auricle. In normothermic controls, anesthesia was continued throughout the experiments. In the hypothermic groups, infusion was terminated when cooling was started, because hypothermia created anesthesia and reduced drug metabolism; infusion was resumed during rewarming at 34°C.

**Respiratory Support**

The rat was placed on the operating table in a supine position. The trachea was opened, and a tracheal tube was inserted. Ventilation was spontaneous and sufficient in all animals at core temperatures >20°C. At <20°C, respiration was supported by a volume-controlled small-animal respirator (NEMI, Medway) using room air. A pH-stat strategy, achieved by adjusting ventilation in accordance with blood gas analysis (17), was utilized when ventilation was supported during experiments. During rewarming, spontaneous respiration resumed at 20°C core temperature, and respiratory support was discontinued.

**Core Cooling and Rewarming**

The animals in the hypothermic groups were cooled and rewarmed by circulation of cold or warm water (thermostated type RTE-110 water bath, Neslab Instruments, Newington, NH) through U-shaped polyethylene tubes placed in the esophagus and lower bowel, as well as circulation of temperature-adjusted water in a double-layered hollow-aluminum operating table. Core temperature was continuously monitored using thermocouple wires positioned in the aortic arch via the right femoral artery and connected to a thermocouple controller (Thermoalert TH-5, Columbus Instruments, Columbus, OH).

**Hemodynamic Measurements**

Mean arterial pressure (MAP) was obtained through a 22-gauge fluid-filled catheter introduced through the left femoral artery and extended to the aortic bifurcation. Left ventricular (LV) pressure was measured through a 20-gauge fluid-filled catheter inserted into the LV via the right common carotid artery. Catheters were connected to transducers (Transpac III, Abbott, North Chicago, IL), and the signals from the transducers were amplified to 0–10 V and passed to a 12-bit analog-to-digital converter (model BNC 2090, National Instruments, Austin, TX). Signal processing and analysis were performed with a computer program developed in our department using LabVIEW version 6.0 (National Instruments). LV pressure was differentiated to obtain the maximum rate of rise in LV pressure (dP/dt max). CO was measured by the thermodilution technique, a method that is reliable also at low core temperatures (13, 23). After saline (0.1–0.15 ml) precooled in ice water was injected through the intravenous line positioned in the right auricle and temperature change was recorded by a thermocouple extended to the ascending aorta via the right femoral artery, thermocouple curves were recorded on a Linear-corder (type Mark II, WR3101, Watanabe Instruments). These curves were digitalized with a digitizing table (model 23180, Calcomp Digitizer Products Division, Anaheim, CA), and CO was calculated with a program designed with the LabVIEW package. Pressures and heart rate (HR) were averaged over 10 consecutive heartbeats, and CO was the mean of 3 measurements. The exact positions of the catheters and the thermocouple were verified at autopsy.

**Measurement of Blood Gases and Calculation of V$_{O2}$**

At different temperatures, blood samples were drawn simultaneously from the aorta and the vena cava for determination of Hb, Hct, and blood gases. Venous blood was sampled from a catheter inserted into the right femoral vein and extended to the proximal part of the vena cava. Blood gases and pH were determined using an analyzer (Rapidlab 800, Chiron Diagnostics). All blood samples were analyzed at 37°C. In agreement with Ashwood et al. (1), pH and blood gas values from hypothermic animals were not temperature corrected, except for use of the Rosenthal factor to adjust ventilation to achieve pH 7.4 (pH stat) during stable hypothermia (30). Hb O$_2$ saturation (S$_{O2}$) was calculated from PO$_2$ after correction for pH and base deficit using the nomograms of Kelman and Numm (16). The O$_2$ content of blood was calculated according to the following formula: S$_{O2}$ × Hb × (1.36 × 10$^{-2}$) (ml O$_2$/100 ml blood). V$_{O2}$ was calculated as the product of CO and arteriovenous difference in O$_2$ content (a-v O$_2$ difference).

**CK-MB and Myocardial Tissue and Plasma Lactate**

CK-MB was measured in plasma samples collected at the end of the experiment from hypothermic animals and normothermic controls. Analysis was performed at Cobas Mira S (Roche Diagnostic Systems, Basel, Switzerland) using a commercially available diagnostic reagent (ABX Pentra CK-MB RTU, ABX Diagnostics, Montpellier, France). Results are presented as a percentage of the mean values of the normothermic control group. At the end of the experiment, the hearts were exteriorized through a sternotomy and quickly freeze clamped with Wollenberger clamps precooled in liquid nitrogen. Then they were vacuum dry frosted for 24 h (Christ Alpha 1-4, Medizinischer Apparatbau, Osterode, Harz, Germany) and pulverized for 3 min at 1,600 rpm by a microdisembrator (Braun Messungen). Myocardial tissue lactate was measured in myocardial homogenate extract by the Cobas Fara II Chemistry System (Roche Diagnostics, Mannheim, Germany) using a lactate analysis kit (catalog no. 1822837, Roche Diagnostics, Indianapolis, IN). Plasma lactate from blood samples was measured using the same method.

**Western Blot of HSP70**

To assess the effect of hypothermia and rewarming on the levels of HSP70 in the heart tissue, 20 mg of freeze-dried heart tissue were lysed by three 5-s periods of sonication in 200 μl of lysis buffer.
containing 150 mmol/l NaCl, 10 mmol/l Tris·HCl, pH 7.5, 2 mmol/l EDTA, 1% (vol/vol) Triton X-100, and Complete protease inhibitor cocktail (Roche). After sonication, the samples were mixed with appropriate volumes of SDS gel loading buffer, boiled for 5 min, and centrifuged (17,000 g at 4°C). The proteins were resolved using 10% polyacrylamide gels and transferred to nitrocellulose membranes. For detection of HSP70-immunopositive bands, membranes were blocked in 5% nonfat dry milk for 1 h at room temperature, washed three times in Tris-buffered saline containing 0.1% Tween 20, and incubated with mouse monoclonal anti-HSP70 antibodies (Stressgen Bioreagents) at 1:1,000 dilution for 1 h at room temperature. After they were washed, the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibodies (0.5 μg/ml; Transduction Laboratories, Lexington, KY) and visualized with a chemiluminescence-based detection system. For statistical analysis, densitometry data of immunopositive bands at 70 kDa were analyzed using Lumi-Analyist software (Boehringer Mannheim). Equal protein load of the gels was controlled by immunoblotting the membranes against GAPDH antibodies (Sigma-Aldrich, St. Louis, MO) and secondary goat anti-rabbit antibodies (0.5 μg/ml; Transduction Laboratories).

Mean pixel density values of immunopositive bands in groups of seven hearts each were checked for statistically significant differences. Results are presented as a percentage of the mean values of the normothermic control group.

**Blood Volume Determination**

Blood volume was determined at different temperatures by the method of Tschaikowsky et al. (35), in which hydroxyl ethyl starch (HES) is used as a dilution marker. Blood (0.4 ml) was drawn from the arterial line just before injection of 0.5 ml of HES into the venous line and again 5 min after the injection. Hct was determined 5 min after injection of HES into blood that had been centrifuged (Centri A 13, Jouan, Saint Nazaire, France) for 5 min at 12,000 rpm. From the same blood samples, plasma glucose levels were determined. In accordance with the method of Tschaikowsky et al. (35), concentrated HCl was added to plasma samples to hydrolyze glucose from HES. Plasma glucose was determined by the Cobas Fara II Chemistry System using a glucose kit (catalog no. 1447513, Roche Diagnostics). Total blood volume (BV) was determined as follows: BV = 3,082 × VolHES/Δglucose/(1 – Hct), where Δglucose is the difference in plasma glucose levels before and after HES (mg%), 3,082 (mg%) is a standard factor given by Tschaikowsky et al. (35), and VolHES is the volume of HES injected (ml).

**Experimental Design**

After surgery, the animals were allowed to rest for 40 min before the start of the experiment. The rats were assigned to three groups: a normothermic control group (group 1) and two hypothermic groups (groups 2 and 3). Animals in group 1 (n = 7) were kept at 37°C for 5 h, those in group 2 (n = 7) were kept at 15°C for 1 h and rewarmed, and those in group 3 (n = 7) were kept at 15°C for 5 h and rewarmed. Duration of cooling and rewarming (~1 h) was equal.

**Measurements**

**Hemodynamics.** Hemodynamics were measured at 37°C, 34°C, 28°C, 24°C, 20°C, and 15°C in groups 2 and 3. During stable hypothermia, the same variables (with the exception of CO) were recorded every 60 min. In group 1, hemodynamic variables were recorded after stabilization and then every 60 min.

**Blood gases.** Blood gas measurements were obtained at 37°C, 28°C, and 20°C, at the start of stable hypothermia (T0), and before rewarmin (T1 and T5) in groups 2 and 3 and every 60 min in group 1.

**Blood volume.** Blood volume was measured before cooling, before rewarmin, and after rewarmin in groups 2 and 3 and after stabilization and then after 3 and 5 h at 37°C in group 1.

**RESULTS**

During rewarmin, one animal in group 2 (at 34°C) and two animals in group 3 (at 36°C and 37°C) died. However, results from seven rats from each group are presented. In the nonsurviving animals, a reduction of CO preceded a sudden fall in MAP, while spontaneous respiration stopped. Results from these animals are not included.

**Core Cooling**

**Hemodynamics.** Except for LV end-diastolic pressure (not shown) and total peripheral resistance (TPR), which remained unchanged, and stroke volume (SV), which increased significantly, all hemodynamic variables were depressed by cooling to 15°C in groups 2 and 3 (Fig. 1).

**O2 supply and VO2.** In response to cooling to 20°C, the calculated values for tissue O2 supply and VO2 appeared linearly reduced (see Fig. 3B).

**Stable Hypothermia (15°C)***

Because of technical limitations, CO could not be measured below 20°C. Therefore, calculations depending on SV and TPR, as well as O2 supply and VO2, are not available below 20°C.

**Hemodynamics.** During 1 h of hypothermia (15°C) in group 2, no significant changes in HR (not shown), MAP, or LV dP/dtmax were found compared with corresponding values obtained when 15°C was reached during cooling (T0).

After 5 h of hypothermia (15°C) in group 3, LV dP/dtmax was significantly reduced. HR (not shown) and MAP remained unchanged during 5 h of hypothermia (15°C).

**Arteriovenous O2 difference.** During 1 h of hypothermia (15°C) in group 2, a-v O2 difference remained constant (Fig. 2, C and D). In contrast, a significant increase in a-v O2 difference...
in group 3 indicates a change in the balance between $O_2$ transport and $O_2$ extraction during 5 h of hypothermia (15°C).

**Rewarming**

**Hemodynamics.** During rewarming from 1 h of hypothermia (15°C) in group 2, CO and most other hemodynamic variables returned to control (Fig. 1). The exception was SV, which was significantly lowered after rewarming.

In contrast, after rewarming from 5 h of hypothermia (15°C) in group 3, HR, CO, SV, and LV $dP/dt_{max}$ were significantly lower than their prehypothermic control values. During rewarming of animals from 24°C in group 3, CO remained significantly lower than corresponding values in group 2. HR and MAP were significantly lower in group 3 than in group 2 at 34°C. In group 3, SV was significantly decreased compared with group 2 from 34°C to 37°C. During rewarming from 5 h...
of hypothermia (15°C) in group 3, TPR was significantly higher than corresponding values in group 2 between 24°C and 34°C. At 34°C, TPR was temporarily significantly elevated compared with control in both groups, but, after rewarming, TPR returned to prehypothermic control values. This fact may well be related to hemodynamic effects of pentobarbital sodium, inasmuch as infusion (7.5 mg·kg⁻¹·h⁻¹) was resumed at 34°C to achieve adequate anesthesia and to create equal anesthetic conditions at 37°C, with effects on hemodynamic function.

**O2 supply and V˙O2.** V˙O2 increased linearly during rewarming and returned to levels measured during cooling in groups 2 and 3 (Fig. 3, A and B). Similarly, after normothermia was reestablished, V˙O2 returned to within prehypothermic control values.

In contrast to 1 h of hypothermia (15°C) in group 2, during which O2 supply was maintained during cooling as well as rewarming, which is also indicated by a maintained a-v O2 difference (Fig. 3A), O2 supply was significantly lowered during rewarming after 5 h of hypothermia (15°C) in group 3. By extraction of more O2 from Hb, as indicated by a significant increase in a-v O2 difference in group 3, V˙O2 was maintained during rewarming after 5 h of hypothermia (15°C).

**HSP70 and CK-MB.** HSP70 content in myocardial tissue from rats rewarmed after 1 h of hypothermia (15°C) in group 2 was not different from that in myocardial tissue from group 1. In contrast, in group 3, HSP70 content increased significantly after exposure to 5 h of hypothermia (15°C) compared with groups 1 and 2 (Fig. 4A).

Activity of CK-MB after rewarming from 5 h of hypothermia (15°C) in group 3 increased more than twofold compared with groups 1 and 2. There were no differences in activity of CK-MB between groups 1 and 2 (Fig. 4B).

**Circulating blood volume.** Circulating blood volume was reduced by 60% after 1 h at 15°C in group 2 and reduced further (by 65%) after the next 4 h at 15°C in group 3 (Fig. 5). After animals in both groups were rewarmed, circulating blood volume increased almost twofold but remained significantly lower than the corresponding prehypothermic control levels.

**Blood gases, pH, Hb, and Hct during cooling and rewarming.** Arterial PO2 remained within physiological levels in animals during spontaneous room air breathing (Table 1). Increased solubility for O2 and CO2 at low temperatures resulted in increases in Po2 and Pco2 during hypothermia. During stable hypothermia and controlled ventilation, the pH-stat strategy was followed. As 15°C was reached during cooling, pH was 7.1 (T0), which gives pH 7.4 when corrected for temperature (30). However, in group 2 after 1 h of hypothermia (15°C, T1) and in group 3 after 5 h of hypothermia (15°C), pH fell to 7.0 without a change in arterial Pco2. After rewarming, a mild-to-
moderate metabolic acidosis in group 3 was associated with spontaneous hyperventilation.
Although significantly changed, the differences in Hb and Hct were modest in groups 2 and 3 throughout the experimental period.

Plasma and myocardial tissue lactate level. Plasma and myocardial tissue lactate level was unchanged after 1 h of hypothermia (15°C) in group 2 compared with corresponding values in group 1. In contrast, lactate level in plasma as well as in myocardial tissue was significantly elevated after rewarming from 5 h of hypothermia (15°C) in group 3 compared with groups 1 and 2 (Fig. 6).

DISCUSSION
The present experiments demonstrate that although CO was restored in animals after rewarming from 1 h of profound hypothermia (15°C), CO was substantially reduced after rewarming subsequent to 5 h of hypothermia (15°C). This indicates that duration of exposure to profound hypothermia is an essential factor in restitution of CO and, therefore, O2 transport during rewarming. Furthermore, mechanisms facilitating unloading of O2 from Hb to compensate for a compromised O2 supply are maintained during rewarming from profound hypothermia. This conclusion is based on the findings that 1) after rewarming subsequent to 1 h, as well as 5 h, of hypothermia (15°C), whole body VO2 returns to within prehypothermic control levels and 2) corresponding values for VO2 during cooling and rewarming are not different between the two groups.

Tissue O2 Supply
O2 supply and VO2 depend on numerous factors of which many, if not all, are spontaneously changed by a decrease in core temperature. Among these factors are metabolic rate, CO, regional blood flow, pH, blood viscosity, O2 solubility, shift in O2-Hb dissociation curve, circulating blood volume, and Hb concentration. Different pH strategies are being used to optimize O2 transport during hypothermia. However, despite controversies (10, 17, 34, 40) regarding “ideal” pH strategies during clinical hypothermia, lactate accumulation is commonly observed during hypothermia and, also, cardiopulmonary bypass, although this method is utilized to optimize tissue blood flow and O2 transport (3, 4, 22, 25, 29). This suggests inadequate oxygenation or metabolic distress in at least some tissues, despite optimized techniques. As temperature decreases, more O2 will be dissolved in blood at a given partial pressure. It has been demonstrated that below 25°C tissue metabolic requirements for O2 decrease and dissolved O2 is increasingly

Fig. 3. Arteriovenous O2 difference (A), O2 supply and oxygen consumption (B), and arterial and venous O2 content (C) in groups 2 and 3 during cooling and after rewarming. Values are means ± SD. *Significantly different from baseline (P < 0.05). +Significantly different from group 2 (P < 0.05).
able to meet those requirements (39). This corresponds with observations during cooling from 28°C in our animals showing a linear drop in a-v O₂ difference, indicating excess O₂ availability. Several authors have described effects of temperature on the human O₂-Hb dissociation curve (2, 9, 11, 12). In the present experiments, pH was maintained at 7.4 (pH-stat strategy) during stable hypothermia (<20°C), when respiration was supported, to induce a rightward shift of the O₂-Hb dissociation curve and facilitate (theoretically) O₂ dissociation from Hb at the tissue level. An attempt to elevate O₂ concentration in inspired air (inspiratory fraction of O₂) in this situation is reported to have a limited effect on O₂ transport (19), and an inspiratory fraction of O₂ of 0.21 (room air) was used in the present experiments. Arterial SO₂ remained stable at 97–100% throughout the experiments, also indicating that hypothermia did not hamper pulmonary gas exchange. At 15°C, a 10–15% reduction of Hb as well as Hct was measured, most likely due to reduction of circulating blood volume when red blood cells and, to a lesser degree, plasma that were stuck in capillaries were excluded from circulation during hypothermia. Several investigators observed that hypothermia induces intravascular platelet aggregation, sludging, and subsequent capillary occlusion (15, 20). During stable hypothermia, only a modest increase in Hb and Hct occurred, and these values were practically reversed after rewarming. Thus limited O₂ supply in circulated tissue due to anemia could be excluded. Using the present model and a similar protocol for hypothermia that does not require blood donors, we previously reported that Hb and Hct remain unaltered by cooling and rewarming (38).

Because of technical limitations, only arterial and venous O₂ content could be measured during stable hypothermia (15°C). Despite optimal arterial O₂ content, a significant reduction in venous O₂ content was found. There are no reports in the literature stating that metabolism, or Q₁₀, is changing during stable hypothermia; therefore, the increase in a-v O₂ difference in our experiments indicates that O₂ supply is reduced in this period. This assumption is supported by Popovic and Kent (28), who reported a continuous reduction of CO in rats during the late phase of stable, profound hypothermia (9–10 h at 15°C). They also reported that total body V₀₂ was first reduced after 6–7 h, whereas, during the first 5 h, increased O₂ extraction was assumed to compensate for the reduction of circulatory function.
Reduced circulating blood volume combined with a stable Hb and Hct during the experiments indicates that whole blood is excluded from circulation during profound hypothermia. In previous experiments using the present model (38), we demonstrated that organ blood flow during hypothermia was almost uniformly reduced to only a fraction of that measured before cooling was initiated. This leads to the assumption that blood is trapped in some vascular beds at least during hypothermia. It is also well known that cooling increases blood viscosity (8, 21). Viscosity was not measured in the present experiments, but one may anticipate that increased viscosity is an important underlying factor in blood flow stagnation and, thus, trapping of blood during hypothermia. This phenomenon is reported to take place when core temperature is lowered (21, 33). The metabolic acidosis observed after rewarming from 5 h of hypothermia (15°C) supports the assumption that some tissue is deprived of circulation during hypothermia but partly reperfused during rewarming. Reduction of circulating blood volume (>60%) during the early phase of cooling or stable hypothermia is new information and is clearly demonstrated in our experiments showing only a modest (5%) further decrease in blood volume after 5 h at 15°C.

As a biological marker of cell stress or cell injury related to graded exposure to hypothermia, we measured myocardial tissue HSP70 after rewarming subsequent to 1 h as well as 5 h of hypothermia (15°C). In the present experiments, we found a significant increase in myocardial HSP70 after 5 h of hypothermia (15°C) but not after rewarming from 1 h of profound hypothermia. To the best of our knowledge, this is the first time that an increase of HSP70 has been demonstrated in myocardial tissue from the in situ beating heart after exposure to graded hypothermia (31). Our finding is supported by experiments in vitro by Bes et al. (5), who reported excess HSP70 production after rewarming of isolated cardiomyocytes prepared from neonatal rat hearts after 2.5 and 4 h of cold storage at 8°C, in contrast to cardiomyocytes rewarmed after only 1 h of storage. Our finding of elevated myocardial tissue HSP70 simultaneously with elevated plasma CK-MB and reduced myocardial mechanical function further supports the hypothesis that HSP70 is a marker of cell stress after exposure to long-term (5 h) hypothermia and rewarming. One may further suggest that increased expression of HSP70 is possibly a natural protective response of the cell to deleterious effects of lowering core temperature. Thus hypothermia-induced protein denaturation is a potential trigger for the induction of heat shock proteins (HSP70).
shock proteins, which are required for refolding of malfolded or denatured proteins.

**Reduced Myocardial Mechanical Function**

Pathophysiological mechanisms to explain posthypothermic reduction of myocardial function remain unsettled. The present experiments focusing on O2 transport and VO2 allow us to conclude that depressed CO is unrelated to myocardial hypoxia during hypothermia and rewarming. The modest increase in plasma lactate during rewarming most likely has its origin in tissues other than the heart, more likely in hypoperfused or nonperfused tissues during hypothermia (see above). Our interpretation is that, after several hours of hypothermia, lactate accumulated in hypoperfused tissues and was washed out during rewarming, when some of these tissues were reperfused. This interpretation is also supported by our previous measurements of coronary sinus blood flow during rewarming in dogs (36). Rewarming from 5 h of mild-to-moderate hypothermia increased total plasma lactate significantly, but the lactate was not of cardiac origin (36). The modest increase in myocardial tissue lactate after 5 h of hypothermia (15°C) in the present experiments is most likely a consequence of increased lactate consumption due to its increased availability as a metabolic substrate. Metabolic acidosis, a consequence of lactate production in the present experiments, is known to reduce cardiac function. However, an argument to exclude acidosis as a cause of cardiac dysfunction in the present experiments is that spontaneous normalization of pH simultaneously with a depression of myocardial function to a level even greater than in the present experiments has previously been shown after rewarming in the actual model using a similar protocol (37). With respect to effects of reduced circulating blood volume on LV function after rewarming, comparison of the two experimental groups yields interesting information. In contrast to rats rewarmed after 1 h of profound hypothermia, in which hemodynamic variables spontaneously returned to prehypothermic control, CO remained significantly lowered during, as well as after, rewarming from 5 h of hypothermia (15°C). Inasmuch as circulating blood volume and the index of cardiac preload, LV end-diastolic pressure, are equally lowered in the two groups, reduction in preload does not explain low CO in the animals exposed to 5 h of hypothermia. A factor in CO depression in group 3 is a higher TPR than in group 2. The demonstration in a recent study using the present model that a low afterload was a prerequisite for an increase in CO during rewarming supports the idea that hypothermia induces cardiac failure (18). The elevation of SV in rat hearts in response to hypothermia also shown in the present experiments is consistent with results from other in vivo as well as in vitro experiments. Mechanisms have been proposed in a previous report using the present model (37).

**Study Limitations**

Use of a small-animal model limits the volume and number of blood samples during experiments. Also, because of technical limitations, only venous blood, not coronary sinus blood, could be collected. However, because we obtained whole body VO2 data from two different animal groups exposed to graded hypothermia (1 h vs. 5 h) and rewarming, combined with myocardial tissue metabolic data, we are confident that we can draw conclusions related to myocardial O2 supply. The method for determining blood volume (35) has not been used previously in hypothermic conditions. However, by comparing blood volume changes in two animal groups at the same temperatures, potential methodological errors related to temperature effects are ameliorated. CK-MB has been measured as a marker of myocardial cell damage, because it is commonly used for this purpose. However, all surrogate markers must be considered with caution. Troponins are markers of cell membrane dysfunction, but effects of hypothermia on troponins are less well known.

In conclusion, the present experiments provide information about at least two essential factors concerning O2 supply during rewarming from hypothermia. 1) Because physiological mechanisms that facilitate unloading of O2 from Hb to compensate for compromised O2 transport are maintained during deep/profound hypothermia, O2 supply was not a limiting factor for survival in the present experiments. 2) We observed a hypothermia-induced myocardial dysfunction, demonstrated as a lowering of CO to levels that may seriously compromise O2 supply during rewarming in general. Thus efforts aimed at elevating CO in this phase seem advisable to optimize O2 supply, reperfuse vascular beds, and prevent rewarming shock.

**ACKNOWLEDGMENTS**

We thank Fredrik Bergheim and Knut Steines for technical support in sampling and analysis of hemodynamic data and Elisabeth Børde for assistance in analysis of the plasma samples.

**GRANTS**

This study was supported by a grant from the Laerdal Foundation for Acute Medicine.

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