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RESEARCH PAPER

Maintaining intravenous volume mitigates hypothermia-induced myocardial dysfunction and accumulation of intracellular Ca^{2+}

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Abstract

Previous research exploring pathophysiological mechanisms underlying circulatory collapse after rewarming victims of severe accidental hypothermia has documented post-hypothermic cardiac dysfunction and hypothermia-induced elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in myocardial cells. The aim of the present study was to examine if maintaining euvolaemia during rewarming mitigates cardiac dysfunction and/or normalizes elevated myocardial $[Ca^{2+}]_i$. A total of 21 male Wistar rats (300 g) were surface cooled to 15°C, then maintained at 15°C for 4 h, and subsequently rewarmed to 37°C. The rats were randomly assigned to one of three groups: (1) non-intervention control (n = 7), (2) dextran treated (i.v. 12 ml/kg dextran 70; n = 7), or (3) crystalloid treated (24 ml/kg 0.9% i.v. saline; n = 7). Infusions occurred during the first 30 min of rewarming. Arterial blood pressure, stroke volume (SV), cardiac output (CO), contractility (dP/dt_{max}) and blood gas changes were measured. Post-hypothermic changes in $[Ca^{2+}]_i$ were measured using the method of radiolabelled Ca^{2+} ($^{45}Ca^{2+}$). Untreated controls displayed post-hypothermic cardiac dysfunction with significantly reduced CO, SV and dP/dt_{max}. In contrast, rats receiving crystalloid or dextran treatment showed a return to pre-hypothermic control levels of CO and SV after rewarming, with the dextran group displaying significantly better amelioration of post-hypothermic cardiac dysfunction than the crystalloid group. Compared to the post-hypothermic increase in myocardial $[Ca^{2+}]_i$ in non-treated controls, $[Ca^{2+}]_i$ values with crystalloid and dextran did not increase to the same extent after rewarming. Volume replacement with crystalloid or dextran during rewarming abolishes post-hypothermic cardiac dysfunction, and partially mitigates the hypothermia-induced elevation of $[Ca^{2+}]_i$.

KEYWORDS
microcirculation, rewarming shock, volume replacement

1 | INTRODUCTION

Successful rewarming of patients after accidental hypothermia is often complicated by hypothermia-induced myocardial dysfunction, clinically ranging from a minor depression of cardiac output (CO) to a fulminant circulatory collapse ('rewarming shock') (Maclean & Emslie-Smith, 1977; Tveita, 2000). Hypothermia-induced myocardial dysfunction presents as a left ventricular systolic dysfunction during and after rewarming (Filseth et al., 2010). The pathophysiological mechanisms are not completely understood, but preclinical experiments have revealed that at least part of the dysfunction is caused by impairment of the contractile apparatus within cardiomyocytes. In addition, significant elevation of intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) takes place during hypothermia (Wold et al., 2013), and it remains elevated after rewarming (Kondratiev et al., 2008; Wold et al., 2013).

Depending on the depth and severity of hypothermic exposure, cooling and rewarming can disrupt a number of physiological processes. For example, in response to cooling, there are changes in circulatory parameters, which include a progressive reduction of heart rate (HR), mean arterial pressure (MAP) and CO (Filseth et al., 2010; Tveita et al., 1996). Furthermore, there is a profound increase in blood viscosity during hypothermia, which gives rise to a marked elevation of total peripheral resistance (TPR) that is aggravated by a simultaneous increase in vascular tone (Brown et al., 2012). The resulting low-flow state induced by cooling disrupts shear forces and can lead to intravascular aggregation of red blood cells, which has been demonstrated in hypothermic microcirculation (Grossman & Lewis, 1964; Lofstrom, 1959). These red blood cell aggregates can become lodged at the entrance to capillaries and block flow through individual micro-vessels, impairing effective circulation as red blood cells are sequestered in peripheral tissues (Lipowsky, 2005). Along with a hypothermia-induced impairment of the vascular barrier and a subsequent increase in fluid extravasation from the intravascular to the interstitial space (Hammersborg et al., 2005), there may be a significant loss of plasma volume and circulating blood volume in the hypothermic patient.

It remains unclear whether hypovolaemia is an essential factor in rewarming shock (Tveita, 2000). Based on the observation that hypothermia-induced loss of plasma volume and circulating blood volume may reverse upon rewarming, some have advocated caution against administering large volumes of fluid to accidental hypothermia patients (Lloyd, 1996). However, there is preclinical evidence that fluid loss does not necessarily resolve, especially after prolonged hypothermic exposure (Kondratiev et al., 2006; Tveita et al., 1996). Thus, to avoid intravascular hypovolaemia during rewarming, fluid loss must be compensated by fluid administration, and often in considerable amounts (Brown et al., 2012; Farstad & Husby, 2014; Paal et al., 2016; Truhlar et al., 2015). Still, there is a lack of consensus concerning the type of fluid to be given, with some recommending liberal use of warm crystalloid solutions (Brown et al., 2012), while others routinely administer colloid solutions only during rewarming from severe hypothermia (Farstad et al., 2006; Suominen et al., 2010). Compared to crystalloid solutions, administering colloids during rewarming from hypothermia is associated with improved post-hypothermic haemodynamic function, and reported to limit oedema formation and total fluid requirements (Farstad & Husby, 2014). Dextrans, specifically, are demonstrated to counteract the formation of red blood cell aggregates in the hypothermic microcirculation (Lofstrom, 1959).

In a rat model of hypothermia–rewarming shock, we previously observed post-hypothermic reductions in CO and stroke volume (SV), as well as a 15–20% loss of circulating blood volume after rewarming (Kondratiev et al., 2006). In the present study, we hypothesized that post-hypothermic myocardial dysfunction and elevation of [Ca^{2+}]$_i$ is mitigated by maintaining euvolaemia during rewarming, and that the use of colloids could have beneficial haemodynamic effects surpassing crystalloid solutions.

New Findings

- **What is the central question of this study?**
  Detailed guidelines for volume replacement to counteract hypothermia-induced intravascular fluid loss are lacking. Evidence suggests colloids might have beneficial effects compared to crystalloids. Are central haemodynamic function and level of hypothermia-induced calcium overload, as a marker of cardiac injury, restored by fluid substitution during rewarming, and are colloids favourable to crystalloids?
- **What is the main finding and its importance?**
  Infusion with crystalloid or dextran during rewarming abolished post-hypothermic cardiac dysfunction, and partially mitigated myocardial calcium overload. The effects of volume replacement to support haemodynamic function are comparable to those using potent cardio-active drugs. These findings underline the importance of applying intravascular volume replacement to maintain euvolaemia during rewarming.

2 | METHODS

2.1 | Ethical approval

Adult male Wistar rats (250–350 g; Harlan UK Ltd, UK) were used in the present study. The experimental protocol was approved by the Norwegian Animal Research Authority (ref. no.: 08/62182-1) in accordance with the recommendations of the Federation of European Laboratory Animal Science Associations. On arrival, the animals were

quarantined for 1 week, provided *ad libitum* access to food and water, and housed in accordance with guidelines for accommodation and care of animals (article 5 of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986).

2.2 | Anaesthesia

Anaesthesia was induced by an i.p. injection of 50 mg/kg pentobarbital sodium, followed by a continuous infusion of 7.5 mg/kg/h through an intravenous line in the right jugular vein, extended to the right auricle. Due to hypothermia-induced anaesthesia and reduced drug metabolism, infusion was terminated at temperatures <30°C during cooling, and reintroduced at 30°C during rewarming. Animals were continuously monitored by toe-pinch for any sign of discomfort, and additional anaesthesia was provided if necessary. No neuromuscular blockers were used at any time during the experiment. After rewarming to 37°C and subsequent data sampling, animals were euthanised by an i.v. injection of 1 ml pentobarbital sodium (50 mg/ml).

2.3 | Respiratory support

The rats were placed on an operating table in the supine position. The trachea was incised, and a 14 G tracheal tube inserted. All animals had spontaneous and sufficient ventilation (monitored by P_{aCO_2}) at core temperatures >20°C. At core temperatures <20°C, normo-ventilation (P_{aCO_2}, 5.18–6.39 kPa) was achieved by a volume-controlled small-animal respirator (New England rodent ventilator, model 141, New England Instruments, Medway, MA, USA) using room air.

2.4 | Core cooling and rewarming

Animals were cooled and rewarmed by circulation of cold or warm water (recirculating water bath heater, RTE-110, Neslab Instruments, Newington, NH, USA) through U-shaped polyethylene tubes placed in the oesophagus and the lower bowels. Also, water from the same water bath circulated through the double layered operating table made of hollow aluminium. Core temperature was continuously monitored using a thermocouple wire positioned in the aortic arch via the right femoral artery, connected to a thermocouple controller (Thermoalert TH-5, Columbus Instruments, Columbus, OH, USA).

2.5 | Haemodynamic measurements

Previously, we used a pressure–volume conductance catheter to monitor left ventricular cardiac function. However, in the present experiment, as a consequence of infusing relatively large intravenous volumes, significant changes in the electrical conductance of blood precluded reliable volume measurements using this conductance cather. Therefore, CO was measured using the thermodilution technique, first described by Fegler (1954), by injecting 0.1–0.15 ml of 0.9% saline precooled in ice water through an intravenous line positioned in the right auricle. The change in temperature was recorded from the thermocouple positioned in the aortic arch. Thermodilution signals were recorded on a Linearcorder (Mark II, WR3101, Watanabe Instruments, Tokyo, Japan), digitalized (at 1 kHz sampling rate) using a Calcomp digitizing table (model 23180, Calcomp Digitzer Products Division, Anaheim, CA, USA) and analysed without further signal processing. CO was calculated according to the method described by Hanwell & Linzell (1972), with a program designed with the LabView package (LabVIEW 6.0, National Instruments, Austin, TX, USA) and calculated as the mean of three consecutive measurements.

A 22 G, fluid-filled catheter was placed in the left femoral artery for continuous recording of arterial pressure. The signals from the blood pressure transducer were amplified and digitized (12-bit analog-to-digital converter; BNC 2090, National Instruments) at a 1 kHz sampling rate. Signal processing and data analysis were performed with the help of a unique computer program developed at our department using a LabView package.

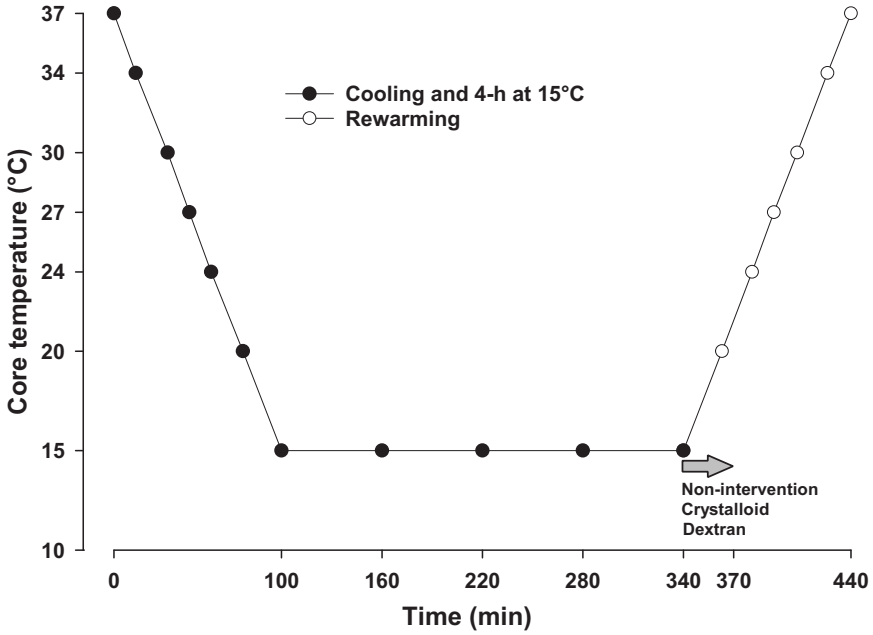
2.6 | Blood gases and acid–base parameters

Blood gases, O_2 saturation, pH and base excess were measured in 0.15 ml arterial blood samples taken from the femoral artery at the start of the experiment, at 15°C, and after rewarming to 37°C. Samples were analysed by a RapidLab 800 blood gas analyser (Chiron Diagnostics, Emeryville, CA, USA).

2.7 | Measurement of $[Ca^{2+}]_i$

Total myocardial $[Ca^{2+}]_i$ was measured using a method previously described in detail (Kondratiev et al., 2008), which was based on the incorporation of radiolabelled Ca^{2+} ($^{45}Ca^{2+}$) and adapted to an *in vivo* experiment. In brief, 20 μCi of $^{45}Ca^{2+}$ (ARX-102 Calcium-45, American Raidolabeled Chemicals Inc., St Louis, MO, USA) was injected at the start of the experiment. Pilot experiments revealed a rapid reduction of $^{45}Ca^{2+}$ activity in the plasma, reaching a steady state level by 120 min after injection. In order to wash out extracellular $^{45}Ca^{2+}$ in the myocardium, the hearts were excised and perfused in a Langendorff system with Krebs–Hensleit bicarbonate buffer containing 11.1 mM glucose and 2.4 mM Ca^{2+} at room temperature. We found that extracellular $^{45}Ca^{2+}$ was washed out after 1 min, and a washout period of 3 min was chosen, after which the hearts were freeze clamped, vacuum dry frosted (Christ Alpha 1–4; Medizinischer Apparatebau, Osterode, Harz, Germany) and subsequently pulverized by a micro-dismembrator (Braun Messungen AG, Melsungen, Germany). In the homogenate, 80–90 mg was extracted in perchloric acid, centrifuged at 7000g (Kubota 1700 centrifuge; Cubota Corp., Tokyo, Japan), and the $^{45}Ca^{2+}$ activity in the supernatant was determined. To determine the specific activity of the isotope, an arterial blood sample, drawn immediately before terminating the experiment, was centrifuged at 9000g, and the $^{45}Ca^{2+}$ activity and Ca^{2+} concentration in plasma were determined

FIGURE 1 Experimental protocol



using a liquid scintillation spectrometer (Model 1900 TR, Packard Instrument Co., Downers Grove, IL, USA) and RapidLab 800 blood gas analyser (Chiron Diagnostics Corp.) respectively. Ultimately, $[Ca^{2+}]_i$ was calculated from the tissue radioactivity, the specific activity of the plasma and the dry weight of the heart tissue. As this method cannot distinguish between intracellular Ca^{2+} compartments, that is, cytosolic, sarcoplasmic reticulum and mitochondrial, only total myocardial $[Ca^{2+}]_i$ was measured.

2.8 | Blood volume determination

Blood volume was determined at the end of the experiment using the method described by Tschaikowsky et al. (1997), 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. Haematocrit was determined 5 min after injection of HES that had been centrifuged at 11400g (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 to the method of Tschaikowsky et al. (1997), concentrated HCl was added to plasma samples to hydrolyse glucose from HES. Plasma glucose was determined by the Cobas Fara II Chemistry System using a glucose kit (Roche Diagnostics, Basel, Switzerland). Total blood volume (BV) was determined as follows: $BV = 3082 \times Vol_{HES}/\Delta glucose/(1 - Hct)$, where Δglucose is the difference in plasma glucose levels before and after HES (mg%), 3082 (mg%) is a standard factor given by Tschaikowsky et al. (1997), and Vol_{HES} is the volume of HES injected (ml).

After surgical instrumentation, animals were allowed to rest for 45 min before starting the experiment and obtaining baseline measurements. After cooling and the 4-h period at 15°C, animals were randomized into one of three experimental groups (Figure 1):

Group 1 ($n = 7$), non-intervention control. The animals were cooled from 37°C to 15°C during a 100-min period, maintained at 15°C for 4 h, and then rewarmed over a 100-min period before being euthanised. No intravenous fluids were given except the fluids accompanying anaesthesia.

Group 2 ($n = 7$), dextran treated. The animals were cooled from 37°C to 15°C during a 100-min period, maintained at 15°C for 4 h, and then rewarmed over a 100-min period before being euthanised. During the rewarming period, these animals were given an i.v. infusion of 12 ml/kg dextran 70 (60 mg/ml dextran in 0.9% saline) in addition to the fluids accompanying anaesthesia.

Group 3 ($n = 7$), crystalloid treated. The animals were cooled from 37°C to 15°C during a 100-min period, maintained at 15°C for 4 h, and then rewarmed over a 100-min period before being euthanised. During the rewarming period, these animals were given an i.v. infusion of 25 ml/kg 0.9% saline, in addition to the fluids accompanying anaesthesia.

2.9 | Calculations

Stroke volume (SV) was calculated as: CO/HR. TPR was calculated as: MAP/CO.

2.10 | Statistics

Results are presented as means and SD. Hemodynamic variables in Figure 4 and 5, and myocardial $[Ca^{2+}]_i$ values in Figure 6 are presented as median with interquartile range, 10th and 90th

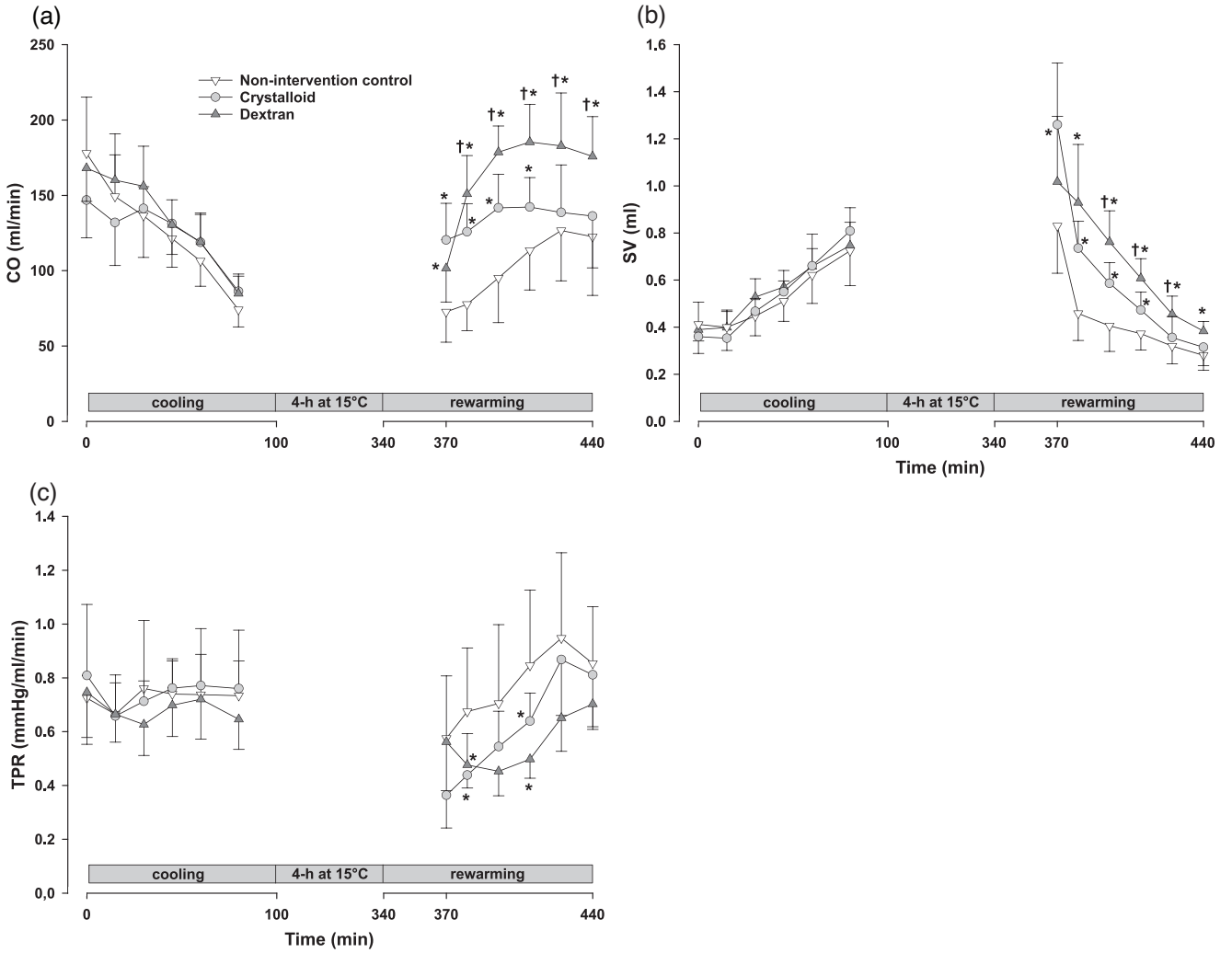


FIGURE 2 Haemodynamic function during hypothermia and rewarming. (a) Cardiac output (CO); (b) stroke volume (SV); (c) total peripheral resistance (TPR). Values are means ± SD. Each group $n = 7$; * $P < 0.05$ vs. corresponding value in the non-intervention control group; †$P < 0.05$ vs. corresponding value in the crystalloid group

percentile. For between-group comparisons of haemodynamic variables and myocardial $[Ca^{2+}]_i$, a one-way ANOVA was used. When significant differences were found, *P*-values were obtained by using Scheffe's test in hypothermic groups. For within-group comparisons of normothermic baseline against post-hypothermic end point, a paired Student's *t*-test was used. To compare variables measured in plasma samples, a two-way RM ANOVA with Dunnett's *post hoc* test was used. Differences were considered significant at $P < 0.05$.

3 | RESULTS

3.1 | Haemodynamic function (Figures 2 and 3)

As in previous studies using this animal model (Haheim et al., 2017; Wold et al., 2013), we found that haemodynamic function was stable during normothermic conditions. There were no differences in pre-hypothermic baseline haemodynamic values among the three groups.

3.1.1 | Cooling and 4 h at 15°C

Compared to pre-hypothermic baseline, cooling to 15°C caused a reduction in most haemodynamic variables. Due to technical limitations related to both the conductance catheter and thermodilution techniques, CO could not be measured below 20°C, and consequently, calculations of TPR and SV could not be made below this temperature. At 20°C a substantial reduction in CO was measured (~50%; Figure 2a), whereas TPR remained unchanged (Figure 2c), and SV was increased (~200%; Figure 2b). At 15°C the following haemodynamic variables were substantially reduced: HR (~87%; Figure 3a), MAP (~57%; Figure 3b), dP/dt_{max} (~88%; Figure 3c) and dP/dt_{min} (~93%; Figure 3d). No further changes were measured in

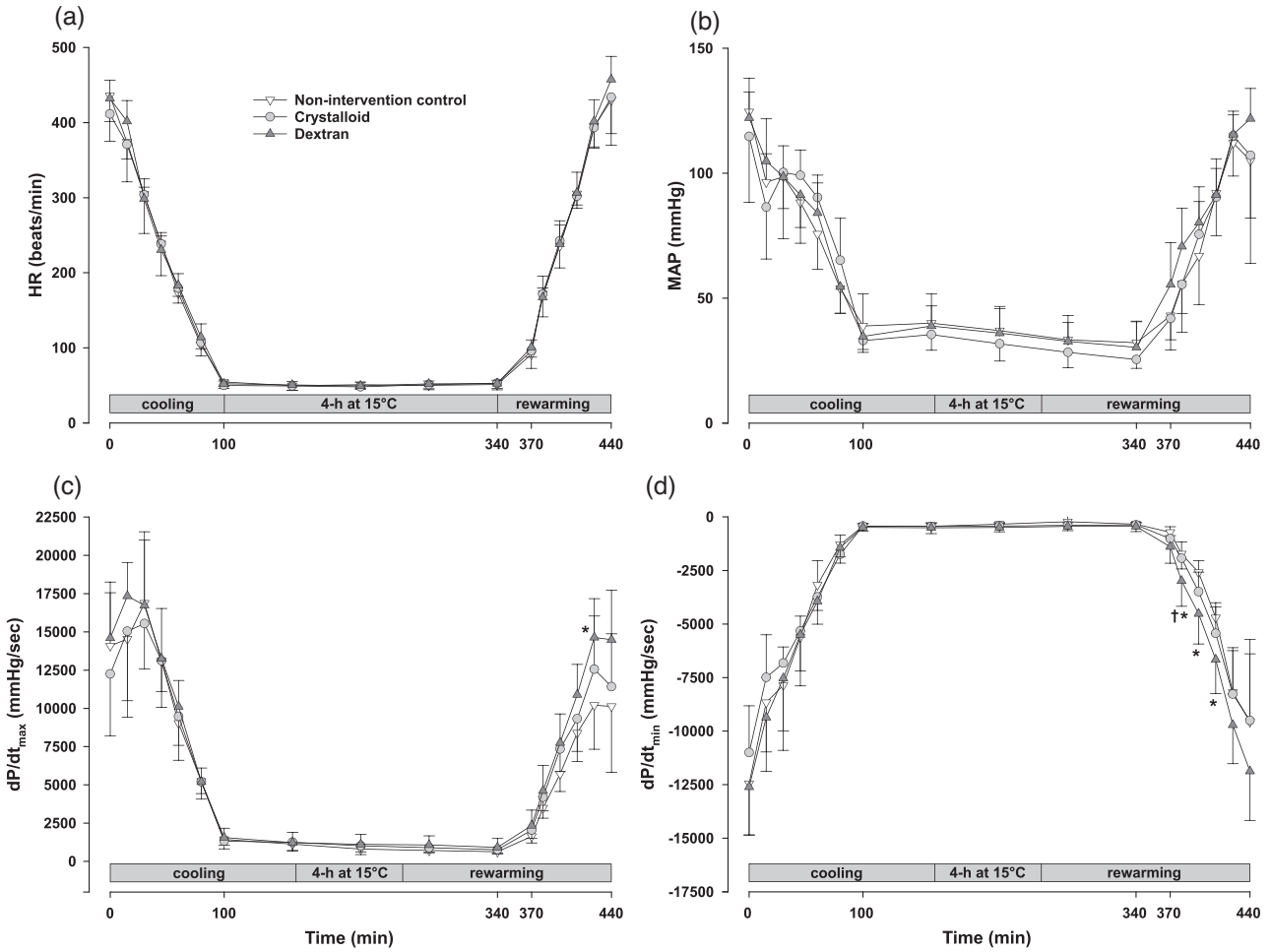


FIGURE 3 Haemodynamic function during hypothermia and rewarming (cont.). (a) Heart rate (HR); (b) mean arterial pressure (MAP); (c) maximum rate of LV pressure rise (dP/dt_{max}); (d) maximum rate of LV pressure decline (dP/dt_{min}). Values are means ± SD. Each group, $n = 7$; * $P < 0.05$ compared to corresponding value in the non-intervention control group; † $P < 0.05$ compared to corresponding value in the crystalloid group

any of the haemodynamic variables during 4-h maintenance of core temperature at 15°C.

3.1.2 | Rewarming to 37°C

Comparisons among groups

In the dextran-treated group, CO was significantly increased compared to both the crystalloid-treated and the non-intervention groups, and remained elevated throughout rewarming to 37°C (Figure 2a). In response to cooling and rewarming, HR underwent substantial changes (Figure 3a), but there was no differences among groups in HR, and therefore, the increase in CO in response to dextran was due to the significant increase in SV (Figure 2b), over that of the two other groups, during rewarming. In contrast, in the crystalloid-treated group, there was a significant increases in CO and SV compared to the non-intervention group, but these effects lasted only half way through the 100 min rewarming period (Figure 2a, b).

3.1.3 | Pre-hypothermic versus post-hypothermic differences (Figures 4 and 5)

In contrast to the non-intervention group, where there were significant reductions in CO (Figure 4a), SV (Figure 4b), and the index of left ventricular contractility, dP/dt_{max} (Figure 5c) after rewarming, all of these haemodynamic variables returned to pre-hypothermic baseline values after rewarming in both treatment groups.

3.2 | Post-hypothermic myocardial $[Ca^{2+}]_i$ (Figure 6)

Compared to the non-intervention control group, post-hypothermic myocardial $[Ca^{2+}]_i$ was significantly lower in both the crystalloid-treated (−47%) and the dextran-treated (−49%) groups.

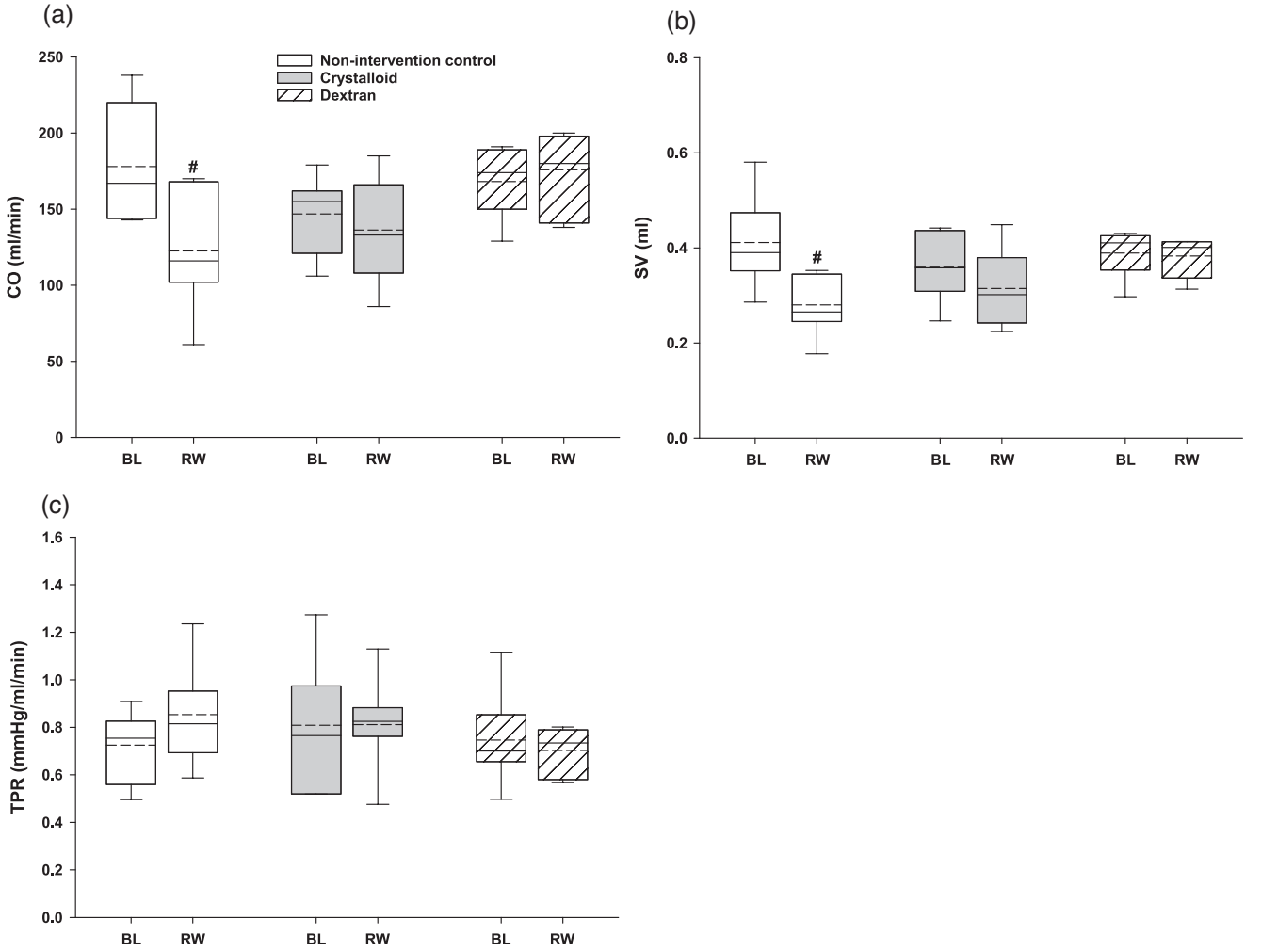


FIGURE 4 Pre-hypothermic vs. post-hypothermic haemodynamic function. (a) Cardiac output (CO); (b) stroke volume (SV); (c) total peripheral resistance (TPR). BL, pre-hypothermic baseline; RW, after rewarming to 37°C. Each group, $n = 7$. Values are presented as vertical boxes with median (solid line), mean (dashed line), interquartile range with 10th and 90th percentile error bars. #$P < 0.05$ vs. intragroup pre-hypothermic baseline

3.3 | Post-hypothermic arterial gas levels (Table 1)

Compared to their corresponding pre-hypothermic values, cooling to 15°C was associated with a significant reduction in pH in all groups. In the crystalloid-treated group, there was an elevation of P_{aCO_2}, but within physiological levels. Base excess (BE) was lower in the crystalloid and dextran-treated groups compared to the non-intervention control group.

After rewarming, when compared to their corresponding pre-hypothermic control values, animals in all groups demonstrated a significant increase in serum lactate levels in concert with reduced BE and pH, and a compensatory hyperventilation. Blood volume was measured in the non-intervention control group and the crystalloid-treated group only, but no differences between the two groups were found after rewarming. There were no differences among groups in post-hypothermic levels of serum cardiac troponin I. However, these levels were elevated (7–10 times) when compared to levels previously reported for normothermic time-matched control animals (Dietrichs et al., 2014). This suggests that hypothermia/rewarming induces cardiac tissue damage in this model.

4 | DISCUSSION

This study demonstrated that intravenous volume replacement, using crystalloid or dextran treatment during rewarming from hypothermia, significantly improved post-hypothermic haemodynamic function and mitigated the hypothermia-induced elevation of myocardial $[Ca^{2+}]_i$. This is in contrast to non-intervention control animals in which hypothermia/rewarming induced reductions in SV, CO, dP/dt_{max}, and significantly higher $[Ca^{2+}]_i$. In the crystalloid-treated group, the effects of volume replacement to support haemodynamic function was limited to the period of fluid administration, whereas in the dextran-treated

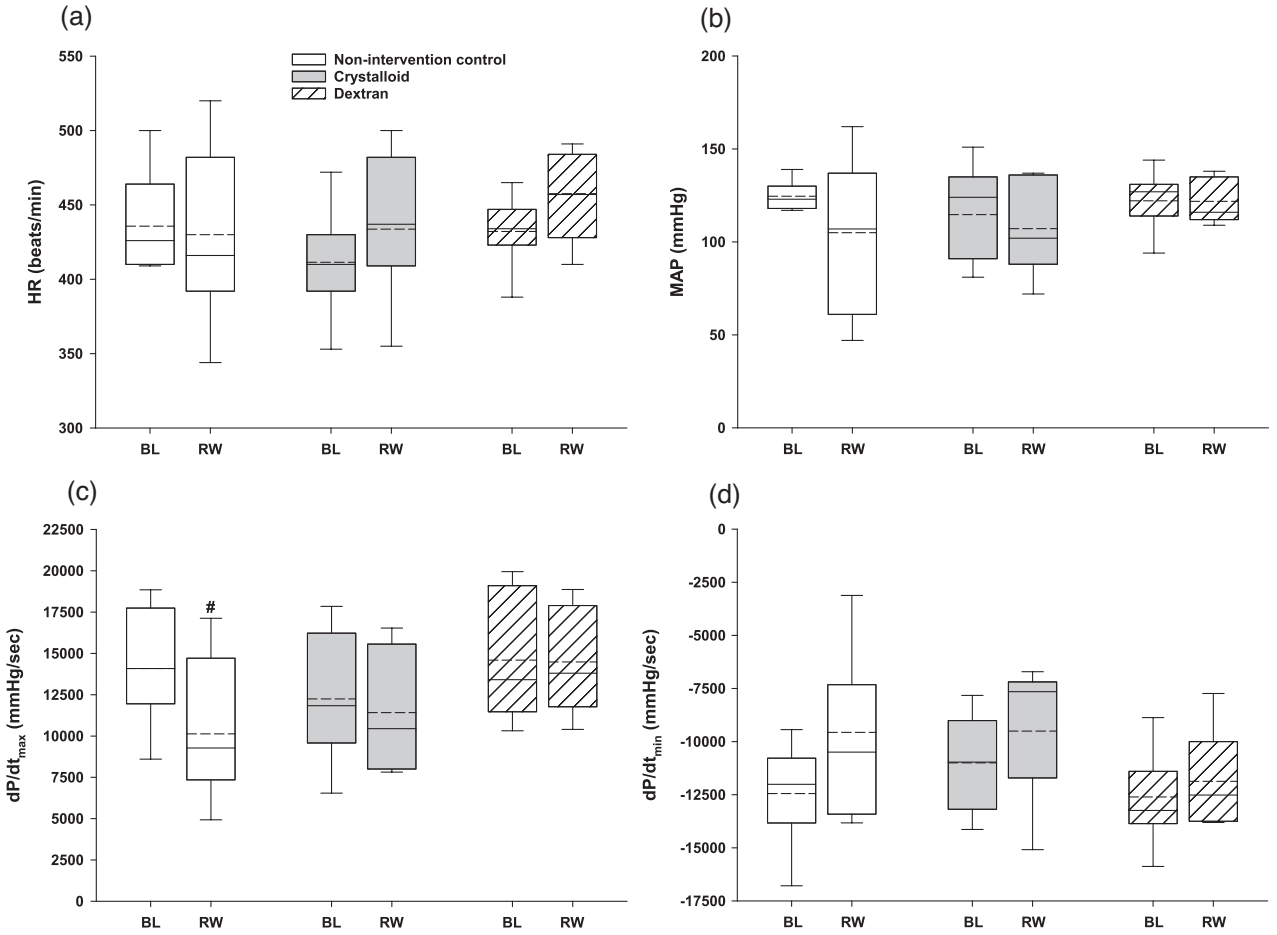


FIGURE 5 Pre-hypothermic vs. post-hypothermic haemodynamic function (cont.). (a) Heart rate (HR); (b) mean arterial pressure (MAP); (c) maximum rate of LV pressure rise (dP/dt_{max}); (d) maximum rate of LV pressure decline (dP/dt_{min}). Each group, $n = 7$. Values are are presented as vertical boxes with median (solid line), mean (dashed line), interquartile range with 10th and 90th percentile error bars. #$P < 0.05$ vs. intragroup pre-hypothermic baseline

group the improved haemodynamic function remained throughout rewarming.

The effects of volume replacement during rewarming to elevate cardiac mechanical function and contractility are comparable to those of previous experiments documented in response to pharmacological interventions (Dietrichs et al., 2014; Kondratiev et al., 2006; Tveita & Sieck, 2012). However, in this study the actual intervention protocol prevented us from using the conductance catheter, which is otherwise routinely used in this experimental model. Therefore, continuous detailed information about left ventricular pressure/volume changes in response to volume infusions could not be monitored and this challenged our detailed interpretation of causal effects of this treatment.

Rewarming from hypothermia and reperfusion after hypo-perfusion or ischaemia during normothermia share the same treatment strategy: restoration of macro-vascular perfusion in an attempt to optimize micro-vascular blood flow. Essential determinants of micro-vascular blood flow are plasma viscosity, haematocrit, red blood cell deformability and red blood cell aggregation (Surgenor, 2013). All of these determinants are seriously affected during low-flow hypothermia. As a consequence, rewarming is often challenged by a marked elevation of SVR (Brown et al., 2012), microvascular aggregation of red blood cells (Grossman & Lewis, 1964; Lipowsky, 2005; Lofstrom, 1959) and fluid extravasation (Hammersborg et al., 2005), causing plasma volume loss and subsequent reduction of circulating blood volume (Chen & Chien, 1978; Farstad et al., 2003). The presence of red blood cell aggregates creates a situation of heterogeneous micro-vascular blood flow where perfused capillaries appear in close proximity to non-perfused capillaries (Lofstrom, 1959; Svanes, 1966), causing organ hypoxia despite normalized global O_2 transport and CO during rewarming.

In the present study, a plausible explanation for the effects of intravenous volume replacement during rewarming in mitigating reduced haemodynamic function appears straightforward. However, a causal relationship between intravenous volume replacement and the mitigation of hypothermia/rewarming-induced myocardial $[Ca^{2+}]_i$ overload is not as obvious. In animals receiving crystalloid or dextran treatment, the increased circulating blood volume will increase venous return thereby increasing preload, which will subsequently elevate SV and improve contractility via the Frank–Starling mechanism

TABLE 1 Variables measured in plasma samples

Parameter	Group	Pre-hypothermic	15°C	Post-hypothermic
pH	Control	7.36 (0.05)	7.24 (0.07)#	7.20 (0.06)#
	Crystalloid	7.35 (0.02)	7.18 (0.07)#	7.21 (0.06)#
	Dextran	7.33 (0.03)	7.21 (0.08)#	7.26 (0.03)#
P_{aCO_2} (kPa)	Control	4.0 (0.66)	5.0 (0.88)	2.8 (0.36)
	Crystalloid	4.4 (0.42)	6.2 (1.67)#	2.9 (0.33)#
	Dextran	4.3 (0.18)	5.4 (1.27)	3.3 (0.65)
P_{aO_2} (kPa)	Control	11.1 (4.1)	22.3 (6.3)#	12.9 (3.6)
	Crystalloid	10.6 (1.3)	26.9 (5.0)#	11.7 (2.1)
	Dextran	10.2 (1.7)	23.4 (5.9)#	10.8 (3.1)
Hb (g/dl)	Control	11.7 (1.2)	11.2 (2.0)	11.3 (1.8)
	Crystalloid	12.8 (1.4)	11.1 (1.5)	11.5 (1.6)
	Dextran	11.3 (1.0)	11.3 (1.1)	9.9 (1.5)
Hct (%)	Control	36.2 (3.7)	36.1 (3.7)	34.8 (5.5)
	Crystalloid	39.2 (4.1)	34.1 (4.4)	35.4 (4.8)
	Dextran	34.8 (3.0)	35.0 (3.1)	31.3 (4.5)
Lactate (mmol/l)	Control	1.3 (0.6)	0.9 (0.4)	3.5 (1.3)#
	Crystalloid	2.0 (1.3)	1.7 (0.8)	4.0 (1.2)#
	Dextran	1.3 (0.9)	1.6 (0.7)	3.7 (2.0)#
BE (mmol/l)	Control	−7.2 (1.7)	−7.0 (7.0)	−18.8 (2.3)#
	Crystalloid	−7.1 (1.5)	−10.6 (2.0)*	−18.3 (2.2)#
	Dextran	−7.9 (1.7)	−11.3 (2.0)#*	−15.1 (2.2)#
cTn-I (ng/ml)	Control	—	—	10 (5.6)
	Crystalloid			14.1 (7.8)
	Dextran			9.8 (5.8)
CBV (ml)	Control ($n = 5$)	—	—	18.7 (5.3)
	Crystalloid ($n = 7$)			20.1 (6.4)
	Dextran			—

Values are means (SD), $n = 7$; *$P < 0.05$ compared to non-intervention control group; #$P < 0.05$ compared to pre-hypothermic baseline. BE, base excess; CBV, circulating blood volume; cTn-I, cardiac troponin I; Hb, haemoglobin; HCT, haematocrit.

(Guyton, 1977). This fundamental property of the heart, by some researchers suggested to be the consequence of increased myofilament Ca^{2+} sensitivity at longer sarcomere lengths (de Tombe et al., 2010), would oppose the reduced Ca^{2+} sensitivity induced by hypothermia–rewarming (Han et al., 2010, 2018; Schaible et al., 2016; Tveita et al., 2019).

During volume infusion, there was a significant increase in SV, CO and heart work, which would provide an increase in coronary blood flow. The absence of an increase in serum lactate levels during rewarming, over that in non-treated control, indicates the presence of a patent coronary autoregulation to provide an adequate myocardial O_2 supply–consumption balance to meet the increased heart work during volume replacement. In this case, volume replacement might have increased myocardial microcirculation, which, in the dextran-treated group, remained throughout the rewarming phase. To speculate, an increase in myocardial micro-vascular blood flow in response to volume infusion also suggests increased clearance of the hypothermia-induced $[Ca^{2+}]_i$ overload. In support of this suggestion is the well-documented (Fukusumi & Adolph, 1970; Grossman & Lewis, 1964; Lofstrom, 1959) positive effect of dextran treatment in preventing hypothermia-induced red blood cell aggregates, which is the background for using dextran in the present experiment.

Intravenous volume replacement during rewarming should preferentially correct intravascular hypovolaemia, restore microcirculatory function, while limiting oedema formation and fluid overload, factors which in clinical medicine are related to increased patient mortality (Chappell et al., 2008).

By adding crystalloid solutions during normothermia, the intravascular volume effect is only about 20%, as crystalloids are evenly distributed throughout the extracellular fluid compartment (Chappell et al., 2008), and this effect may be further reduced by hypothermia (Schanche et al., 2019; Roberts et al., 1985). In contrast, infusing colloid solutions reduces fluid extravasation and oedema formation during hypothermia, and these solutions are routinely administered during

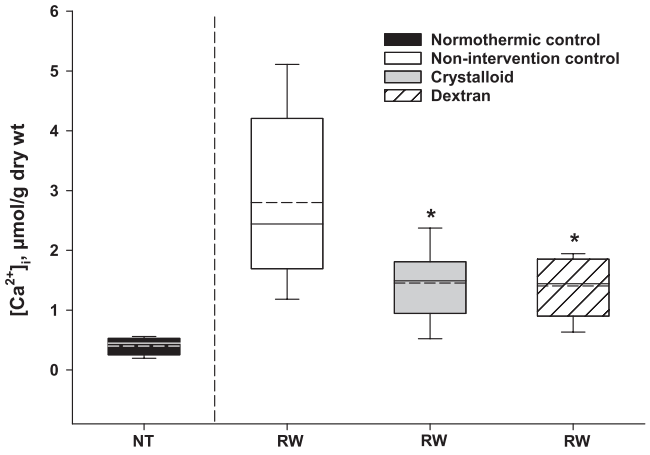


FIGURE 6 Concentration of $[Ca^{2+}]_i$ in cardiac tissue in normothermic controls and after rewarming. Each group, n = 7. Values are presented as vertical boxes with median (solid line), mean (dashed line), interquartile range with 10th and 90th percentile error bars. *$P < 0.05$ vs. non-intervention control. Normothermic control values included are from previous studies to illustrate normothermic baseline levels of $[Ca^{2+}]_i$ concentration (Kondratiev et al., 2008; Wold et al., 2013)

rewarming from severe hypothermia in some institutions (Farstad et al., 2006; Suominen et al., 2010). Still, the fear of potential side effects such as allergic reactions, coagulopathies and risk of kidney injury, has led to restricted use of synthetic colloids in critically ill patients (Reinhart et al., 2012). Several recent studies, including information on normothermic trauma victims and critically ill patients, has shown that a ratio of crystalloids to colloids necessary to achieve the same physiological targets is about 1.5:1 (Annane et al., 2013; Orbegozo et al., 2015; Spahn et al., 2019). Based on this, we chose to use a 2:1 crystalloid to colloid ratio in the present study.

The maintenance of euvolaemia during rewarming in the treatment groups was indicated by the fact that haematocrit levels did not change, that is, there was no evidence of haemodilution. Due to technical limitations, circulating plasma volumes were measured only in the non-intervention control and crystalloid-treated groups, but there was no difference in circulating blood volume between these two groups after rewarming. This may be the consequence of increased extravasation of crystalloids at low core temperatures (Farstad et al., 2005, 2006), limiting the volume effect only to the period of ongoing infusion, also indicated by the temporary mitigating effect of crystalloid treatment on haemodynamic function. The moderate but significant reduction in pH and elevated plasma lactate levels in all groups indicate the absence of massive organ hypoxia during hypothermia/rewarming. In support, we found normal values of global O_2 partial pressure in arterial blood.

In previous studies using animal models of hypothermia/rewarming, we observed time-dependent elevation of myocardial $[Ca^{2+}]_i$ (Kondratiev et al., 2008; Wold et al., 2013). After 30 min at 15°C $[Ca^{2+}]_i$ remained unaltered (Wold et al., 2013), whereas after 4 h at 15°C, there was a more than six-fold increase in $[Ca^{2+}]_i$ compared to pre-hypothermic levels (Wold et al., 2013). After rewarming, myocardial $[Ca^{2+}]_i$ only partially recovered (−15%), but remained substantially increased (Wold et al., 2013). The post-hypothermic elevation of myocardial $[Ca^{2+}]_i$ levels observed in non-intervention control animals in the present study were comparable to those previously reported (Kondratiev et al., 2008; Wold et al., 2013). Importantly, with volume replacement in the treatment groups, myocardial $[Ca^{2+}]_i$ levels were significantly lower after rewarming when compared to the non-intervention control group. Impaired homeostasis of myocardial $[Ca^{2+}]_i$ is a key factor in the pathophysiology of normothermic heart failure (Vassalle & Lin, 2004). In response to hypothermia, there is a decrease in myofilamental Ca^{2+}-sensitivity (Han et al., 2010, 2018; Harrison & Bers, 1989; Schaible et al., 2016; Tveita et al., 2019). These two, seemingly contradictory functional changes, are already present at 30°C (Kusuoka et al., 1991), and the increase in force is associated with an elevation of $[Ca^{2+}]_i$ (Puglisi et al., 1996) in response to cooling. The increase in cytoplasmic $[Ca^{2+}]$ enhances cardiac contractility by increasing the number of cross-bridges recruited for force development, but seemingly, due to a dysfunctional elevation of this ion over time, Ca^{2+} overload occurs (Tani & Neely, 1989; Vassalle & Lin, 2004), which results in mechanical dysfunction that may entail cardiac failure (Aasum & Larsen, 1997; Aasum et al., 1997; Bers et al., 1989; Gambassi et al., 1994; Puglisi et al., 1996; Schiffmann et al., 2001; Shattock & Bers, 1987; Shutt & Howlett, 2008; Steigen et al., 1994; Stowe et al., 1995, 1999, 2000; Groban et al., 2002). Studies using papillary muscle (Han et al., 2010) or isolated cardiomyocytes (Schaible et al., 2016) to investigate excitation–contraction coupling at low temperatures (15°C) have reported that the mechanism for the hypothermia-induced calcium overload over time is related to the prolongation of evoked Ca^{2+} transient in response to stimulation, leaving insufficient time for the evoked transient to return to baseline before the next stimulus. Further, with relevance to outcome after continuous haemodynamic interventions during and after rewarming, we have reported spontaneous recovery of contractile dysfunction and return of calcium overload during a 2-h follow-up period after rewarming in these isolated, perfused and stimulated cells (Schaible et al., 2016).

4.1 | Summary and conclusion

The positive haemodynamic effects were both more pronounced and more protracted with dextran than with crystalloid solution. In addition, we measured significantly lower $[Ca^{2+}]_i$ in cardiac tissue in response to volume replacement, but post-hypothermic levels are still substantially elevated. On this background, we advocate using volume replacement aimed at maintaining euvolaemia during rewarming from long-lasting accidental hypothermia.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Conception or design of the work: J.H.N., T.K., O.H. and T.T. Acquisition, analysis or interpretation of data for the work: J.H.N., T.S., T.K., O.H., G.C.S. and T.T. Drafting of the work or revising it critically for important intellectual content: J.H.N., T.S., T.K., O.H., G.C.S. and T.T. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

All data are available upon reasonable request to the authors.

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