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Ultra-fast and whole body cooling with total liquid ventilation induces favourable neurological and cardiac outcomes following cardiac arrest in rabbits

Short title: Liquid ventilation, hypothermia and cardiac arrest

Chenoune M^{1,2,3}, DVM, MSc; Lidouren F^{1,2,3}, BSc; Adam C⁴, MD; Pons S^{1,2,3}, PharmD, PhD; Darbera L^{1,2,3}, MSc; Bruneval P⁴, MD; Ghaleh B^{1,2,3}, PhD; Zini R^{1,2,3}, PhD; Dubois-Randé J-L^{1,2,3}, MD, PhD; Carli P⁶, MD, PhD; Vivien B⁶, MD, PhD; Ricard J-D⁵, MD, PhD; Berdeaux A^{1,2,3}, MD, PhD, FAHA; Tissier R^{1,2,3}, DVM, PhD.

¹ Inserm, U955, Créteil, 94000, France ;

² Université Paris Est, Faculté de Médecine, Créteil, 94000, France ;

³ Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, 94700, France ;

⁴ Inserm, Unité 970, Paris, 75005, France ;

⁵ Inserm, Unité 722, UFR de Médecine Paris Diderot, Paris, 75018, France ;

⁶ SAMU de Paris, Département d'Anesthésie Réanimation, CHU Necker Enfants Malades, Faculté de Médecine Descartes – Paris V, Paris, 75015, France.

Corresponding author: Renaud Tissier

INSERM, Unité 955, Equipe 3

Faculté de Médecine

8 rue du Général Sarrail

94010 Créteil cedex, France

Tel: +33.1.43.96.73.02 ; Fax: +33.1.43.96.71.34

E-mail: rtissier@vet-alfort.fr

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32 **Abstract**

33 **Background:** In animal models of cardiac arrest, the benefit afforded by hypothermia is
34 closely linked to the rapidity in body temperature decrease after resuscitation. Since total
35 liquid ventilation (TLV) with temperature controlled perfluorocarbons induces a very rapid
36 and generalized cooling, we aimed to determine whether this could limit the post-cardiac
37 arrest syndrome in a rabbit model. We especially focused on neurological, cardiac,
38 pulmonary, liver and kidney dysfunctions. **Methods and Results:** Anesthetized rabbits were
39 submitted to either 5 or 10-min of untreated ventricular fibrillation. After cardiopulmonary
40 resuscitation and resumption of a spontaneous circulation, the animals underwent either
41 normothermic life support (control) or therapeutic hypothermia induced by TLV. The latter
42 procedure decreased esophageal and tympanic temperatures to 32-33°C within only 10-min.
43 After rewarming, the animals submitted to TLV exhibited an attenuated neurological
44 dysfunction and decreased mortality 7 days later as compared to control. The
45 neuroprotective effect of TLV was confirmed by a significant reduction in brain histological
46 damages. We also observed limitation of myocardial necrosis, along with a decrease in
47 troponin I release and a reduced myocardial caspase 3 activity with TLV. The beneficial
48 effects of TLV were directly related to the rapidity at inducing hypothermia since neither
49 conventional cooling (cold saline infusion + external cooling) nor normothermic TLV elicited
50 a similar protection. **Conclusions:** Ultra-fast cooling instituted by TLV exerts potent
51 neurological and cardiac protections in an experimental model of cardiac arrest in rabbits.
52 This could be a relevant approach to afford a global and protective hypothermia against the
53 post-cardiac arrest syndrome.

54

55 **Key Words:** Cardiopulmonary resuscitation; Fibrillation; Heart arrest; Ischemia; Ventilation.

56 **Introduction**

57 Institution of mild “therapeutic” hypothermia (32-34°C) during 24 to 36 hours after
58 resuscitation is known to improve survival and neurological recovery in comatose survivors
59 of cardiac arrest.^{1, 2} However, experimental studies in dogs,^{3, 4} pigs^{5, 6} and rodents^{7, 8}
60 demonstrated that the neuroprotection afforded by hypothermia was related to the rapidity
61 in body temperature decrease after resuscitation. When achieved rapidly, hypothermia
62 could also be beneficial for other organs since it can be, for example, also potentially
63 cardioprotective during myocardial ischemia.⁹⁻¹² Accordingly, many strategies were
64 proposed to afford such a rapid hypothermia, including intravenous infusion of cold fluid,¹³
65 endovascular¹⁴ or intranasal cooling.^{15, 16}

66 Another strategy that can experimentally afford a very rapid and generalized cooling
67 is liquid ventilation of the lungs with temperature-controlled perfluorocarbons.^{11, 17-22} These
68 liquids can use the lungs as heat exchangers while maintaining normal gas exchanges.¹⁸⁻²⁰
69 In addition, this ventilation procedure also protects the lung integrity.²⁰ Using a prototype of
70 total liquid ventilator that alternatively instillates and removes a tidal volume of
71 perfluorocarbon from the lung, we were able to decrease the left atrial temperature to 32°C
72 within only 5 min in anesthetized rabbits.^{11, 17, 18} This was associated with a very potent
73 protection against myocardial infarction and subsequent contractile dysfunction in animal
74 models of coronary artery occlusion.^{11, 17, 18} In a swine model of ventricular fibrillation, liquid
75 ventilation also induced a rapid convective cooling that further improves the chances for
76 subsequent resumption of spontaneous circulation.^{21, 22} However, the effect of hypothermic
77 total liquid ventilation (TLV) has never been investigated to our knowledge in animal models
78 of post-cardiac arrest dysfunction when instituted after resumption of spontaneous
79 circulation.

80 Accordingly, the main purpose of the present study was to investigate the long term
81 effect of ultrafast cooling induced by TLV in a rabbit model of post-cardiac arrest
82 dysfunction following ventricular fibrillation and resuscitation. In order to determine whether
83 hypothermic TLV properly protects through very fast cooling, we investigated two additional

84 groups submitted to a conventional hypothermia (cold saline infusion + external cooling) or
85 to normothermic TLV. The primary outcome was the survival during 7 days of follow-up.
86 The secondary outcomes were clinical, biochemical, hemodynamic and histological
87 parameters describing neurological, cardiac, pulmonary, liver and kidney potential
88 dysfunctions. We also aimed to investigate whether ultra-fast cooling can protect the heart
89 through an early inhibition of cardiac cell death. The latter point was also critical to further
90 support the relevance of very fast cooling to limit the subsequent dysfunction following
91 cardiac arrest.

92 **Methods**

93 The animal instrumentation and ensuing experiments were conducted in accordance
94 with French official regulations (agreement A94-046-13) after approval by the local ethical
95 committee. The investigation conformed to the *Guide for the Care and Use of Laboratory*
96 *Animals* published by the US National Institutes of Health.

97 **Animal preparation**

98 New Zealand rabbits (3.0-3.5 kg) were anesthetized using zolazepam, tiletamine and
99 pentobarbital (all 20-30 mg/kg i.v.). They were intubated and mechanically ventilated. After
100 administration of pancuronium bromide (200 µg/kg i.v.), two electrodes were implanted
101 upon the chest and inserted into the esophagus for subsequent induction of ventricular
102 fibrillation. Rectal, esophageal and tympanic temperatures were continuously monitored
103 using thermal probes (Harvard Apparatus, Paris, France). Throughout the protocol, external
104 electrocardiogram was recorded, as well as arterial blood pressure from a catheter
105 implanted into the ear artery. Data were digitalized and analyzed using the data acquisition
106 software HEM v3.5 (Notocord, Croissy-sur-Seine, France).

107 **Cardiac arrest and cardiopulmonary resuscitation**

108 After animal preparation and subsequent stabilisation, ventricular fibrillation was
109 induced by passing an alternative current (10 V, 4 mA; 2 min) between the implanted
110 electrodes. Mechanical ventilation was stopped at the onset of fibrillation and throughout
111 the subsequent period of cardiac arrest. After either 5 or 10 min of untreated fibrillation,
112 cardiopulmonary resuscitation was started using cardiac massage (~ 200 beats/min),
113 electric attempts of defibrillation (5-10 J/kg) and intravenous administration of epinephrine
114 (15 µg/kg i.v.). Resumption of spontaneous circulation (ROSC) was considered as an
115 organized cardiac rhythm associated with a mean arterial pressure above 40 mmHg during
116 at least 1 min. After ROSC, administration of epinephrine was further permitted during a
117 maximum of 7 h at a dosage appropriately adjusted to maintain mean arterial pressure at
118 ~80 mmHg. Mechanical ventilation was continued until weaning and awakening of the
119 animals. Rabbits subsequently returned to their cage for a survival follow-up. They received

120 antibiotics (enrofloxacin, 5 mg/kg i.m.) during 7 days and analgesics (buprenorphine, 30
121 µg/kg s.c.) during 3 days.

122 Experimental protocol

123 As shown in Figure 1, rabbits randomly underwent either 5 or 10 min of cardiac arrest
124 with subsequent cardiopulmonary resuscitation. For each duration of cardiac arrest, rabbits
125 were randomly allocated to resuscitation under normothermic conditions (Control₅ and
126 Control₁₀ groups, respectively) or with hypothermia induced by TLV (H-TLV₅ and H-TLV₁₀
127 groups, respectively). In these last two groups, TLV was started at the 10th min following
128 cardiopulmonary resuscitation (*i.e.*, after ROSC) by filling the lung with 10 ml/kg of
129 perfluorocarbon (Fluorinert, 3M, Cergy, France) and then connecting the endotracheal tube
130 to our prototype of liquid ventilator (Supplemental Figure 1).^{11, 17, 18} This ventilator was set
131 to a tidal volume of ~7-10 ml/kg of body weight with a respiratory rate of 6 breaths/min. For
132 each breath, the ventilator pumped into and out of the lungs the tidal volume of liquids. The
133 perfluorocarbon mixture was bubbled with 100% O₂. The temperature of the heat
134 exchanger was adjusted to maintain esophageal and tympanic temperatures at a target
135 temperature of ~32°C. After 20 min of TLV and achievement of the hypothermic target
136 temperature, the perfluorocarbon was evacuated from the lungs by gravity and the
137 endotracheal tube was again connected to a conventional mechanical ventilator.
138 Hypothermia was further maintained at 32°C during 3 h, if necessary using cold blankets.
139 Animals were subsequently rewarmed using infra-red lights and thermal pads until weaning
140 from conventional ventilation and awakening. Animals were housed in a closed cage
141 enriched in O₂ during 2-3 days to avoid hypoxic episodes. In order to determine whether
142 hypothermic TLV properly protects through very fast cooling, we investigated two randomly
143 allocated additional groups submitted to 10 min of cardiac arrest. The first of these groups
144 (Saline₁₀) was submitted to 3 h of conventional hypothermia through the combination of
145 cold saline administration (30 ml/kg i.v., NaCl 0.9% at 4°C) and external cooling. The
146 second additional group was submitted to an episode of TLV with normothermic
147 perfluorocarbons (N-TLV₁₀ group) to determine their proper effects.

148 In order to further investigate the effects of hypothermic TLV, additional rabbits were
149 included in the Control_{10'} and H-TLV_{10'} groups, respectively. These animals were
150 euthanized one hour after the cardiac arrest episode for collection of myocardial and blood
151 samples for caspase activity assays and measurement of circulating troponin I,
152 respectively.

153 Neurological and cardiac dysfunction assessment

154 Neurological dysfunction was evaluated daily in surviving animals using a clinical
155 score previously validated in rabbits,²³ as shown in Supplemental Table 1 (0-10% =
156 normal; 100% = brain death). After 7 days of follow-up, surviving rabbits were
157 reanesthetized and a pressure catheter (SciSense, London, Ontario, Canada) was
158 introduced into the left ventricle through the right carotid artery for measurement of end-
159 diastolic pressures as well as positive and negative left ventricular rate of pressure
160 development (dP/dt_{max} and dP/dt_{min}). These parameters were also measured in a group of
161 Sham rabbits that were neither submitted to cardiac arrest nor hypothermia.

162 Blood chemistry and caspase activity assay

163 Blood pH, carbon dioxide and oxygen partial pressures (pCO₂ and pO₂, respectively)
164 were assessed from arterial blood samples with an ABL 77 series analyser (Radio-meter
165 SA, France). Blood lactate was determined on an Accutrend[®] Plus analyser (Roche
166 Diagnostics, Mannheim, Germany). Liver and renal functions were evaluated by measuring
167 the alanine aminotransferase (ALAT) and creatinine concentrations (Prestige 24i, Tokyo-
168 Boehi, Japan). Troponin I and Creatinine Kinase were measured by an off-site laboratory
169 (IDEXX Laboratories, Alfortville, France).

170 Caspase 3 activity was assayed from cardiac samples, as previously described.²⁴
171 Briefly, tissues were homogenized in cold buffer (25 mM HEPES pH 7.5, 5 mM MgCl₂,
172 2 mM EDTA, 0.1% Triton X-100, 2 mM dithiothreitol, 1 mM PMSF, 5 µl/ml protease cocktail
173 inhibitor P8340; Sigma-Aldrich, St Louis, MO, USA). Homogenates were centrifuged and
174 supernatants collected. Proteins (90 µg) were incubated in caspase assay buffer (50 mM
175 HEPES pH 7.4, 100 mM NaCl, 1 mM EDTA, 10 mM dithiothreitol, Triton X-100 0.1%,

176 glycerol 10%). Enzymatic reaction was started by addition of 0.2 mM of the fluorogenic
177 substrates ac-DEVD-AFC (Biomol Research Laboratories, Hambourg, Germany).
178 Fluorescent arbitrary units were converted into pmol/mg protein/h using a standard curve of
179 free AFC (Biomol Research Laboratories, Hambourg, Germany).

180 Histological analyses

181 After 7 days of follow-up after cardiac arrest, the surviving rabbits were finally
182 euthanized for pathological analyses of the heart, lung, kidney, liver and brain. These
183 organs were also removed and analyzed in the animals died before the end of the follow-
184 up. For lungs, a slice was sampled from each lobe (5 per lung). For the heart, we analyzed
185 a mid heart transversal biventricular section. For kidneys, two slices were studied for each
186 organ. We used a 0-3 score system to blindly quantify the severity of each organ alteration,
187 as shown in Supplemental Tables 2 and 3 (0=normal; 3=very severe lesion). The overall
188 brain score was the mean value obtained for cortex, hippocampus and cerebellum, as
189 previously described.²³ For lungs, we assessed two separate scores for cardiogenic lesions
190 (serous edema and/or congestion) and infectious complication of bronchopneumonia,
191 respectively. The first panels of Figures 2 and 3 illustrate typical normal and pathological
192 aspects of the different organs.

193 Statistical analyses

194 Data were expressed as mean \pm SEM. Hemodynamic and biochemical parameters
195 were compared between the different groups and corresponding controls using a two-way
196 ANOVA for repeated measures. Post-hoc analyses were performed at each time-point as
197 compared to controls using a Student t-test with Bonferroni correction. Values were not
198 compared between the different time-points in order to avoid multiple comparisons. In each
199 experimental group, neurological dysfunction and histological scores were compared to the
200 corresponding control group using a Mann-Whitney non parametric test. Survival curves
201 were obtained using a Kaplan-Meier analysis and were compared to the corresponding
202 control group using a log-rank test. These last analyses took only into account the animals
203 that achieved ROSC. Significant differences were determined at $P\leq 0.05$.

204 **Results**

205 Seventy rabbits were included in the present study and submitted to cardiac arrest
206 (n=10, 10, 15, 15, 10 and 10 in the Control_{5'}, H-TLV_{5'}, Control_{10'}, H-TLV_{10'}, Saline_{10'} and N-
207 TLV_{10'} groups, respectively).

208 As shown in Table 1, all rabbits subjected to 5 min of cardiac arrest were successfully
209 resuscitated (Control_{5'} and H-TLV_{5'} groups) whereas only 10/15 were successfully
210 resuscitated in the Control_{10'} and H-TLV_{10'} groups, respectively. This rate was 7/10 in
211 Saline_{10'} and N-TLV_{10'} groups. Regardless the duration of the cardiac arrest, the time to
212 resumption of spontaneous circulation was not significantly different among groups for each
213 duration of cardiac arrest.

214 As illustrated in Figure 4, esophageal, tympanic and rectal temperatures were not
215 significantly different among groups at baseline. A mild and passive decrease in body
216 temperature was observed in the Control_{5'} and Control_{10'} groups after cardiac arrest but this
217 remained within the normothermic range. In H-TLV groups, esophageal and tympanic
218 temperatures decreased very rapidly after the institution of TLV. As example, tympanic
219 temperature achieved 33.3 ± 0.5 and 32.5 ± 0.3 °C in H-TLV_{5'} and H-TLV_{10'} within 10 min after
220 the onset of the cooling protocol, respectively. In the Saline_{10'} group, such tympanic
221 temperatures were achieved after ~30 min. Regarding esophageal and rectal temperatures,
222 the time to achieve 32 to 33°C was less than 5 and 20 min in H-TLV_{10'} whereas this was
223 more than 45 and 60 min in Saline_{10'}, respectively. In the N-TLV_{10'} group, body
224 temperatures did not significantly differ from the Control_{10'} values throughout the
225 experimental protocol.

226 As shown in Table 2, heart rate significantly decreased during the hypothermic phase
227 in hypothermic groups as compared to corresponding controls (e.g., -21%, -28% and -31%
228 at 60 min after cardiac arrest in H-TLV_{5'}, H-TLV_{10'} and Saline_{10'} vs corresponding controls,
229 respectively). Mean arterial pressure was not significantly different between groups
230 throughout the experimental protocol since epinephrine administration was permitted to
231 maintain a ~80 mmHg value during 7 h after cardiac arrest. As shown in Table 1, the total

232 amount of epinephrine administered throughout cardiopulmonary resuscitation was
233 however significantly lower in H-TLV_{10'} vs Control_{10'} (128±128 vs 684±118 µg/kg,
234 respectively), suggesting a favorable hemodynamic effect of hypothermic TLV. We did not
235 observe such a significant difference in H-TLV_{5'} vs Control_{5'} but epinephrine dosages were
236 much lower (174±81 vs 207±58 µg/kg, respectively). In Saline_{10'} and N-TLV_{10'} groups,
237 epinephrine dosages were also not different from Control_{10'}. After discontinuation of any
238 pharmacological support (e.g., at 8 h after cardiac arrest), the lactate levels were
239 significantly lower in H-TLV_{5'} vs Control_{5'} (1.2±0.2 vs 4.8±1.7 mmol/l) and in H-TLV_{10'} vs
240 Control_{10'} (3.6±0.7 vs 7.0±1.7 mmol/l). Those levels were not significantly different among
241 Saline_{10'} and N-TLV_{10'} groups as compared to Control_{10'} (5.9±0.7 and 7.6±0.6 vs 7.0±1.7
242 mmol/l).

243 As shown in Figure 5, we observed a severe acidosis with an increase in pCO₂ and a
244 decrease in pO₂ in all groups following cardiac arrest. In H-TLV_{5'}, pO₂ was lower 15 min
245 after cardiac arrest as compared to Control_{5'}. This could be expected as control animals
246 were ventilated with oxygen whereas TLV rabbits underwent liquid ventilation by that time.
247 At 180 min, gas exchanges were conversely improved in H-TLV groups as compared to
248 controls. As example, blood pH and pO₂ increased whereas pCO₂ decreased in H-TLV_{10'} vs
249 Control_{10'}, respectively. Importantly, all the animals were submitted to conventional
250 ventilation at that time point, with standardized ventilation parameters. As illustrated in
251 Figure 2, the safety of TLV for lungs was also documented by histology demonstrating
252 cardiogenic lesions (serous edema and/or congestion) or infectious complications of
253 bronchopneumonia to a similar extent in TLV groups vs corresponding controls.

254 As shown in Table 2, renal function was not affected after cardiac arrest in all groups
255 since plasma creatinine levels remained within usual values. Conversely, we observed an
256 increase in the liver enzyme ALAT with no difference among TLV and corresponding
257 controls. Kidney and liver lesions were mild with no difference among groups (Figure 2,
258 panel B).

259 As illustrated in Figure 6, neurological dysfunction was significantly attenuated in H-
260 TLV groups as compared to controls. This difference was significant as early as the 2nd day
261 following cardiac arrest in H-TLV_{5'} vs Control_{5'} (panel A) whereas this was observed within
262 24h of follow-up in H-TLV_{10'} vs Control_{10'} (panel B). In Saline_{10'}, a transient improvement in
263 neurological recovery was observed at day 1 but this was no longer significant at day 2
264 after cardiac arrest. As illustrated in Figure 3 (panel B), the neuroprotective effect of
265 hypothermic TLV was further demonstrated by a significant decrease in the severity of the
266 ischemic disorders in the brain in H-TLV_{5'} and H-TLV_{10'} as compared to Control_{5'} and
267 Control_{10'} groups, respectively. No any protection was conversely seen in Saline_{10'} and N-
268 TLV_{10'} as compared to Control_{10'}.

269 A significant difference in survivals was also evidenced between H-TLV groups and
270 corresponding controls, as illustrated in Figure 6 (panels C and D). At the end of the follow-
271 up, 9/10 and 7/10 rabbits survived in the H-TLV_{5'} and H-TLV_{10'} groups as compared to 5/10
272 and 0/10 in Control_{5'} and Control_{10'}, respectively. Conversely, survival was not significantly
273 improved in Saline_{10'} and N-TLV_{10'} as compared to Control_{10'}.

274 As illustrated in Figure 3 (panel B), myocardial foci of necrosis were less frequent in
275 H-TLV_{10'} vs Control_{10'}, demonstrating a cardioprotective effect of hypothermic TLV. No
276 difference was conversely seen between Saline_{10'} and N-TLV_{10'} as compared to Control_{10'}.
277 In surviving animals, the functional myocardial sequels of cardiac arrest were also
278 evaluated after 7 days of follow-up. As shown in Supplemental Table 4, mean blood
279 pressure and heart rate in the conscious state were not different among groups as
280 compared to a Sham group. After anesthesia, end diastolic left ventricular pressure,
281 dP/dt_{max} and dP/dt_{min} were also not different between groups, suggesting that there was
282 no major functional myocardial alterations in those surviving animals.

283 To further explore the cardioprotective effect of hypothermic TLV, eight additional
284 rabbits were included in the Control_{10'} and H-TLV_{10'} groups for a surrogate study dedicated
285 to the caspase activity assays and measurement of troponin I levels. As shown in
286 supplemental Figure 2, troponin I measured 60 min after cardiac arrest significantly

287 decreased in H-TLV_{10'} as compared to Control_{10'} (1.3±0.3 vs 70.7±30.4 ng/ml, respectively).
288 The cardioprotective effect of hypothermic TLV was also supported by a decrease in
289 caspase 3 activity as compared to control (6.2±1.2 vs 10.0±1.2 pmol/mg prot/h,
290 respectively).

291 **Discussion**

292 The present study provides the proof of concept that ultra-fast whole body cooling
293 with hypothermic TLV limits the post-cardiac arrest syndrome when instituted after ROSC in
294 a rabbit model of ventricular fibrillation. Interestingly, we observed potent neuro- and
295 cardioprotections with hypothermic TLV which remains a safe procedure for the lungs. As
296 we used only 3 h of hypothermia, this also suggested that very early hypothermia after
297 ROSC does not need to be prolonged to produce a strong clinical benefit. Importantly, this
298 benefit was directly related to cooling rapidity with TLV since a conventional cooling with
299 cold saline and external blankets was not significantly protective in similar conditions.
300 Proper effects of the perfluorocarbon are unlikely as related to the lack of protection with
301 normothermic TLV.

302 Our first finding is the rapidity of TLV-induced cooling since esophageal and brain
303 temperatures achieved ~32-33°C within only 10 min. In comparison, a conventional
304 hypothermic protocol (cold saline infusion + external cooling) requires ~30 and 45 min to
305 similarly reduce these temperatures. The rapid cooling elicited by TLV was directly related
306 to the tidal exchange of the liquid since simple repetitive pulmonary lavages with a 4°C
307 perfluorocarbon requires more than 60 min to decrease the tympanic temperature to 32°C
308 in the same species.²⁰ In large animals, hypothermic TLV was also reported to afford a very
309 fast cooling and to reduce the pulmonary artery temperature to 32°C within 9-10 min when
310 instituted intra-arrest in a ventricular fibrillation model in swine.²²

311 Importantly, the rapid hypothermia elicited by TLV was associated with a potent
312 neurological protection and an increase in survival rate as compared to control conditions.
313 In animal studies, it is admitted that the neuroprotective effect of hypothermia is time
314 dependent and that a large part of the protection is lost when cooling is delayed.²⁵ For
315 example, in a canine model of cardiac arrest, the neurological protection was lost after only
316 15 min of delay before the onset of hypothermia after ROSC.²⁵ In the present study, we
317 observed a very potent benefit of hypothermia when achieved rapidly after ROSC with TLV
318 whereas a conventional hypothermia was not significantly protective. Recent experiments

319 have also shown that hypothermia started before ROSC (e.g., intra-arrest hypothermia) can
320 afford an additional benefit^{7,8} but this might be difficult to translate this concept into human
321 clinical practice. All these findings demonstrate that most of the possible benefits of
322 hypothermia can be lost within minutes after ROSC, further supporting the need of devices
323 eliciting ultra-fast cooling such as TLV in the present study.

324 Importantly, the benefit of hypothermic TLV observed in our conditions was
325 produced by a short hypothermic episode (3 h), whereas the current recommendations in
326 humans are maintenance of hypothermia during 24-36 h.^{1,2} We choose this short duration
327 since previous experiments have shown that when achieved very early, the duration of the
328 hypothermia does not need to be prolonged to afford an effective neuroprotection, e.g., in a
329 gerbil model of global ischemia.²⁶ Mice studies also noted that 1 h of cooling after ROSC
330 was sufficient to generate a significant clinical benefit.^{7,8} When the severity of the ischemic
331 insult increases or when the onset of cooling is delayed, it is conversely well established
332 that prolonging hypothermia is critical for achieving a maximal neurological protection.^{27,28}
333 As example, a prolonged cooling allowed to provide enduring behavioral and histological
334 protection in rats submitted to permanent middle cerebral artery occlusion, even when
335 delayed after onset of ischemia.²⁷

336 Another important beneficial effect of hypothermic TLV is the cardioprotection
337 observed here like that previously shown in animal models of coronary artery occlusion.^{11,}
338 ^{17,18} This was especially observed after 10 min of cardiac arrest since myocardial lesions
339 were minor in the groups submitted to only 5 min of cardiac arrest. This was evidenced by a
340 limitation in myocardial necrosis and a preservation of myocardial functional performance in
341 surviving rabbits. Cardioprotection was also observed very early after cardiac arrest since
342 troponin I release and caspase 3 activity were significantly decreased within 60 min after
343 resuscitation in H-TLV_{10'} vs Control_{10'}. In animal models of focal myocardial ischemia, the
344 window of protection with hypothermia is virtually limited to the ischemic phase, whereas
345 cooling at reperfusion is ineffective at reducing infarct-size in most experimental studies.¹²
346 In the present study, hypothermia was instituted after global reperfusion (ROSC) but it is

347 reasonable to speculate that the myocardium remains momentarily and partially ischemic
348 even after ROSC. This can explain that a very rapid cooling with TLV can still afford a
349 beneficial effect even if instituted after ROSC and systemic reperfusion. Improved post-
350 resuscitation myocardial function have interestingly also be observed with intra-arrest rapid
351 head cooling.²⁹ Generalized hypothermia could even potentially afford a protection of the
352 liver and/or the kidney.³⁰ As these organs were mildly altered in control conditions in the
353 present study, we were not able to show any difference with hypothermic TLV.

354 Importantly, TLV was a safe procedure for the lungs. Even, we observed improved
355 gas exchanges using standardized ventilatory parameters in TLV vs control groups at 3 h
356 after cardiac arrest. After weaning from ventilation, animals were however maintained in a
357 cage enriched in oxygen to avoid hypoxic episodes.¹¹ In pigs, intra-arrest liquid ventilation
358 was indeed demonstrated to alter lung function since activation of pulmonary macrophages
359 might alter gas exchanges after resumption to conventional ventilation.^{21, 22} In our study, the
360 tolerance of TLV was shown by histological examinations and this is supported by several
361 reports from the literature demonstrating that liquid ventilation can even protect the lungs.^{19,}
362 ²⁰ Several prototypes of liquid ventilator have been developed and the clinical translation of
363 this concept might accordingly be feasible when those devices will be available for a clinical
364 use.³¹ To date, the current prototypes are mostly developed for a paediatric use³¹ and the
365 translation of TLV-induced hypothermia would be accordingly first possible in newborns
366 presenting global ischemia. Further developments might also ultimately permit a translation
367 in adult patients.

368 Our study has several limitations. First, neurological dysfunctions were assessed on
369 the basis of clinical and histological parameters. Other more functional tests or imaging
370 would also be important. Second, histological analyses were performed in all animals,
371 irrespectively of their survival time. This would have lead to an underestimation of the
372 histological scores in some animals who died very early after the cardiac arrest. However,
373 since the lower scores were observed in the group that lived for the longer time (H-TLV₁₀),
374 the latter limitation should not actually impact our conclusions.

375 In conclusion, ultra-fast cooling instituted by hypothermic TLV limits the post-cardiac
376 arrest dysfunction with associated neuro- and cardioprotective effects. Importantly, TLV
377 was a safe procedure for the lungs in our experimental conditions. The beneficial effects of
378 hypothermic TLV were probably directly related to the rapidity in temperature decrease
379 since myocardial cell death inhibition was evidenced even very early following resuscitation.

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396 **Disclosure**

397 None.

398 **References**

- 399 1. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, Smith K.
400 Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia.
401 N Engl J Med. 2002; 346:557-563.
- 402 2. The Hypothermia After Cardiac Arrest Study Group. Mild therapeutic hypothermia to
403 improve the neurologic outcome after cardiac arrest. N Engl J Med. 2002; 346:549-556.
- 404 3. Nozari A, Safar P, Stezoski SW, Wu X, Henschir J, Radovsky A, Hanson K, Klein E,
405 Kochanek PM, Tisherman SA. Mild hypothermia during prolonged cardiopulmonary
406 cerebral resuscitation increases conscious survival in dogs. Crit Care Med. 2004; 32:2110-
407 2116.
- 408 4. Nozari A, Safar P, Stezoski SW, Wu X, Kostelnik S, Radovsky A, Tisherman S,
409 Kochanek PM. Critical time window for intra-arrest cooling with cold saline flush in a dog
410 model of cardiopulmonary resuscitation. Circulation. 2006; 113:2690-2696.
- 411 5. Guan J, Barbut D, Wang H, Li Y, Tsai MS, Sun S, Inderbitzen B, Weil MH, Tang W. A
412 comparison between head cooling begun during cardiopulmonary resuscitation and surface
413 cooling after resuscitation in a pig model of cardiac arrest. Crit Care Med. 2008; 36:S428-
414 433.
- 415 6. Tsai MS, Barbut D, Tang W, Wang H, Guan J, Wang T, Sun S, Inderbitzen B, Weil MH.
416 Rapid head cooling initiated coincident with cardiopulmonary resuscitation improves
417 success of defibrillation and post-resuscitation myocardial function in a porcine model of
418 prolonged cardiac arrest. J Am Coll Cardiol. 2008; 51:1988-1990.
- 419 7. Zhao D, Abella BS, Beiser DG, Alvarado JP, Wang H, Hamann KJ, Hoek TL, Becker
420 LB. Intra-arrest cooling with delayed reperfusion yields higher survival than earlier
421 normothermic resuscitation in a mouse model of cardiac arrest. Resuscitation. 2008;
422 77:242-249.
- 423 8. Abella BS, Zhao D, Alvarado J, Hamann K, Vanden Hoek TL, Becker LB. Intra-arrest
424 cooling improves outcomes in a murine cardiac arrest model. Circulation. 2004; 109:2786-
425 2791.

- 426 9. Hale SL, Dae MW, Kloner RA. Hypothermia during reperfusion limits 'no-reflow' injury
427 in a rabbit model of acute myocardial infarction. *Cardiovasc Res.* 2003; 59:715-722.
- 428 10. Miki T, Swafford AN, Cohen MV, Downey JM. Second window of protection against
429 infarction in conscious rabbits: real or artifactual. *J Mol Cell Cardiol.* 1999; 31:809-816.
- 430 11. Tissier R, Couvreur N, Ghaleh B, Bruneval P, Lidouren F, Morin D, Zini R, Bize A,
431 Chenoune M, Belair MF, Mandet C, Douheret M, Dubois-Rande JL, Parker JC, Cohen MV,
432 Downey JM, Berdeaux A. Rapid cooling preserves the ischaemic myocardium against
433 mitochondrial damage and left ventricular dysfunction. *Cardiovasc Res.* 2009; 83:345-353.
- 434 12. Tissier R, Chenoune M, Ghaleh B, Cohen MV, Downey JM, Berdeaux A. The small
435 chill: mild hypothermia for cardioprotection? *Cardiovasc Res.* 2010; 88:406-414.
- 436 13. Larsson IM, Wallin E, Rubertsson S. Cold saline infusion and ice packs alone are
437 effective in inducing and maintaining therapeutic hypothermia after cardiac arrest.
438 *Resuscitation.* 2010; 81:15-19.
- 439 14. Dixon SR, Whitbourn RJ, Dae MW, Grube E, Sherman W, Schaer GL, Jenkins JS,
440 Baim DS, Gibbons RJ, Kuntz RE, Popma JJ, Nguyen TT, O'Neill WW. Induction of mild
441 systemic hypothermia with endovascular cooling during primary percutaneous coronary
442 intervention for acute myocardial infarction. *J Am Coll Cardiol.* 2002; 40:1928-1934.
- 443 15. Yu T, Barbut D, Ristagno G, Cho JH, Sun S, Li Y, Weil MH, Tang W. Survival and
444 neurological outcomes after nasopharyngeal cooling or peripheral vein cold saline infusion
445 initiated during cardiopulmonary resuscitation in a porcine model of prolonged cardiac
446 arrest. *Crit Care Med.* 2010; 38:916-921.
- 447 16. Boller M, Lampe JW, Katz JM, Barbut D, Becker LB. Feasibility of intra-arrest
448 hypothermia induction: A novel nasopharyngeal approach achieves preferential brain
449 cooling. *Resuscitation.* 2010; 81:1025-1030.
- 450 17. Chenoune M, Lidouren F, Ghaleh B, Couvreur N, Dubois-Rande J-L, Berdeaux A,
451 Tissier R. Rapid cooling of the heart with total liquid ventilation prevents transmural
452 myocardial infarction following prolonged ischemia in rabbits. *Resuscitation.* 2010; 81:359-
453 362.

- 454 18. Tissier R, Hamanaka K, Kuno A, Parker JC, Cohen MV, Downey JM. Total liquid
455 ventilation provides ultra-fast cardioprotective cooling. *J Am Coll Cardiol.* 2007; 49:601-605.
- 456 19. Wolfson MR, Shaffer TH. Pulmonary applications of perfluorochemical liquids:
457 ventilation and beyond. *Paediatr Respir Rev.* 2005; 6:117-127.
- 458 20. Yang SS, Jeng MJ, McShane R, Chen CY, Wolfson MR, Shaffer TH. Cold
459 perfluorochemical-induced hypothermia protects lung integrity in normal rabbits. *Biol*
460 *Neonate.* 2005; 87:60-65.
- 461 21. Riter HG, Brooks LA, Pretorius AM, Ackermann LW, Kerber RE. Intra-arrest
462 hypothermia: both cold liquid ventilation with perfluorocarbons and cold intravenous saline
463 rapidly achieve hypothermia, but only cold liquid ventilation improves resumption of
464 spontaneous circulation. *Resuscitation.* 2009; 80:561-566.
- 465 22. Staffey KS, Dendi R, Brooks LA, Pretorius AM, Ackermann LW, Zamba KD, Dickson E,
466 Kerber RE. Liquid ventilation with perfluorocarbons facilitates resumption of spontaneous
467 circulation in a swine cardiac arrest model. *Resuscitation.* 2008; 78:77-84.
- 468 23. Baker AJ, Zornow MH, Grafe MR, Scheller MS, Skilling SR, Smullin DH, Larson AA.
469 Hypothermia prevents ischemia-induced increases in hippocampal glycine concentrations
470 in rabbits. *Stroke.* 1991; 22:666-673.
- 471 24. Souktani R, Pons S, Guegan C, Bouhidel O, Bruneval P, Zini R, Mandet C, Onteniente
472 B, Berdeaux A, Ghaleh B. Cardioprotection against myocardial infarction with PTD-
473 BIR3/RING, a XIAP mimicking protein. *J Mol Cell Cardiol.* 2009; 46:713-718.
- 474 25. Kuboyama K, Safar P, Radovsky A, Tisherman SA, Stezoski SW, Alexander H. Delay
475 in cooling negates the beneficial effect of mild resuscitative cerebral hypothermia after
476 cardiac arrest in dogs: a prospective, randomized study. *Crit Care Med.* 1993; 21:1348-
477 1358.
- 478 26. Carroll M, Beek O. Protection against hippocampal CA1 cell loss by post-ischemic
479 hypothermia is dependent on delay of initiation and duration. *Metab Brain Dis.* 1992; 7:45-
480 50.

- 481 27. Clark DL, Penner M, Wowk S, Orellana-Jordan I, Colbourne F. Treatments (12 and 48
482 h) with systemic and brain-selective hypothermia techniques after permanent focal cerebral
483 ischemia in rat. *Exp Neurol*. 2009; 220:391-399.
- 484 28. Wu X, Drabek T, Kochanek PM, Henchir J, Stezoski SW, Stezoski J, Cochran K,
485 Garman R, Tisherman SA. Induction of profound hypothermia for emergency preservation
486 and resuscitation allows intact survival after cardiac arrest resulting from prolonged lethal
487 hemorrhage and trauma in dogs. *Circulation*. 2006; 113:1974-1982.
- 488 29. Tsai MS, Barbut D, Wang H, Guan J, Sun S, Inderbitzen B, Weil MH, Tang W. Intra-
489 arrest rapid head cooling improves postresuscitation myocardial function in comparison with
490 delayed postresuscitation surface cooling. *Crit Care Med*. 2008; 36:S434-439.
- 491 30. Kang J, Albadawi H, Casey PJ, Abbruzzese TA, Patel VI, Yoo HJ, Cambria RP,
492 Watkins MT. The effects of systemic hypothermia on a murine model of thoracic aortic
493 ischemia reperfusion. *J Vasc Surg*. 2010; 52:435-443.
- 494 31. Robert R, Micheau P, Avoine O, Beaudry B, Beaulieu A, Walti H. A regulator for
495 pressure-controlled total-liquid ventilation. *IEEE Trans Biomed Eng*. 2010; 57:2267-2276.

496 **Legends of Figures**

497

498 **Figure 1:** Experimental protocol

499 *CA, cardiac arrest; TLV, total liquid ventilation initiated; H-TLV, hypothermic TLV; N-TLV,*
500 *normothermic TLV; Saline, hypothermia induced by intravenous administration of cold*
501 *saline combined to external cooling; ROSC, resumption of spontaneous circulation.*

502

503 **Figure 2:**

504 ***Panel A:*** Examples of normal or pathological histological appearances of the kidney, liver
505 and lungs in the TLV and control groups, respectively. In kidney, lesions consisted in dilated
506 regenerative proximal tubules (arrows, bar=120 μm). In liver, we observed systematized
507 clarification of hepatocytes (arrows, bar=120 μm). In lungs, lesions were congestion and
508 serous edema (arrows in the left lung panel, bar=120 μm) or foci of bronchopneumonia
509 (arrows in the right lung panel, bar=120 μm).

510 ***Panel B:*** Histological scores of alteration in kidney, liver and lungs of rabbits from the
511 different groups. For lungs, we assessed two separate scores for cardiogenic lesions and
512 infection complications, respectively. Open circles represents individual scores and the
513 thick line represents the median value of corresponding group.

514 ** $p < 0.05$ vs corresponding control; TLV, total liquid ventilation; H-TLV, hypothermic TLV;*
515 *N-TLV, normothermic TLV; Saline, hypothermia induced by intravenous administration of*
516 *cold saline combined to external cooling.*

517

518 **Figure 3:**

519 ***Panel A:*** Examples of normal or pathological histological appearances of the brain and the
520 heart in the TLV and control groups, respectively. In brain, ischemic disorders consisted in
521 ischemic pyramidal cells with pycnotic nucleus in the hippocampus (arrows, bar=30 μm), in
522 laminar necrosis of Purkinje cells in the cerebellum (arrows, bar=30 μm) or in numerous

523 ischemic neurons in the cortex (arrows, bar=30 μ m), respectively. In the myocardium, we
524 observed foci of cardiomyocytes necrosis (arrows, bar=120 μ m).

525 **Panel B:** Histological scores of alteration in the brain and heart of rabbits from the different
526 groups. Open circles represents individual scores and the thick line represents the median
527 value of the corresponding group.

528 * $p < 0.05$ vs corresponding control; TLV, total liquid ventilation; H-TLV, hypothermic TLV;
529 N-TLV, normothermic TLV; Saline, hypothermia induced by intravenous administration of
530 cold saline combined to external cooling.

531

532 **Figure 4:** Esophageal, tympanic and rectal temperatures in the different experimental
533 groups.

534 * $p < 0.05$ vs corresponding control; $n = 10$ in each experimental group; TLV, total liquid
535 ventilation; H-TLV, hypothermic TLV; N-TLV, normothermic TLV; Saline, hypothermia
536 induced by intravenous administration of cold saline combined to external cooling.

537

538 **Figure 5:** Blood pH, pCO₂ and pO₂ in the different experimental groups.

539 * $p < 0.05$ vs corresponding control; $n = 10$ in each experimental group; TLV, total liquid
540 ventilation; H-TLV, hypothermic TLV; N-TLV, normothermic TLV; Saline, hypothermia
541 induced by intravenous administration of cold saline combined to external cooling.

542

543 **Figure 6:**

544 **Panels A and B:** Neurological dysfunction scores at days 1, 2 and 7 following resuscitation
545 in the different experimental groups submitted to 5 min or 10 min of cardiac arrest,
546 respectively. Open circles represent individual scores and the thick line represents the
547 median value of the corresponding group. Only animals achieving resumption of
548 spontaneous circulation were included.

549 **Panels C and D:** Kaplan-Meyer survival curves in the different experimental groups
550 submitted to 5 min or 10 min of cardiac arrest, respectively. Only animals achieving
551 resumption of spontaneous circulation were included.
552 * $p < 0.05$ vs corresponding control; TLV, total liquid ventilation; H-TLV, hypothermic TLV; N-
553 TLV, normothermic TLV; Saline, hypothermia induced by intravenous administration of cold
554 saline combined to external cooling.
555

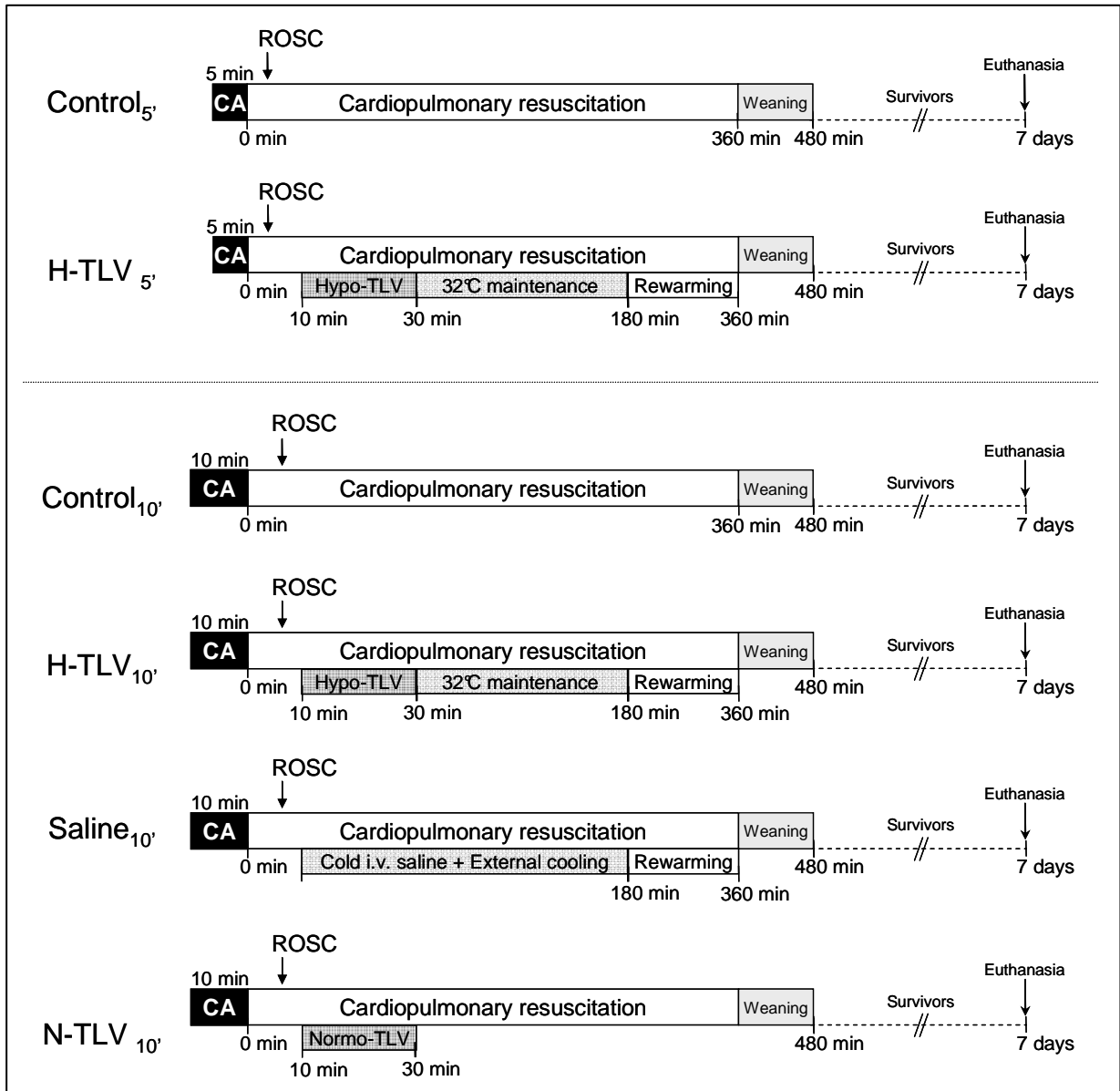
Figure 1

Figure 3

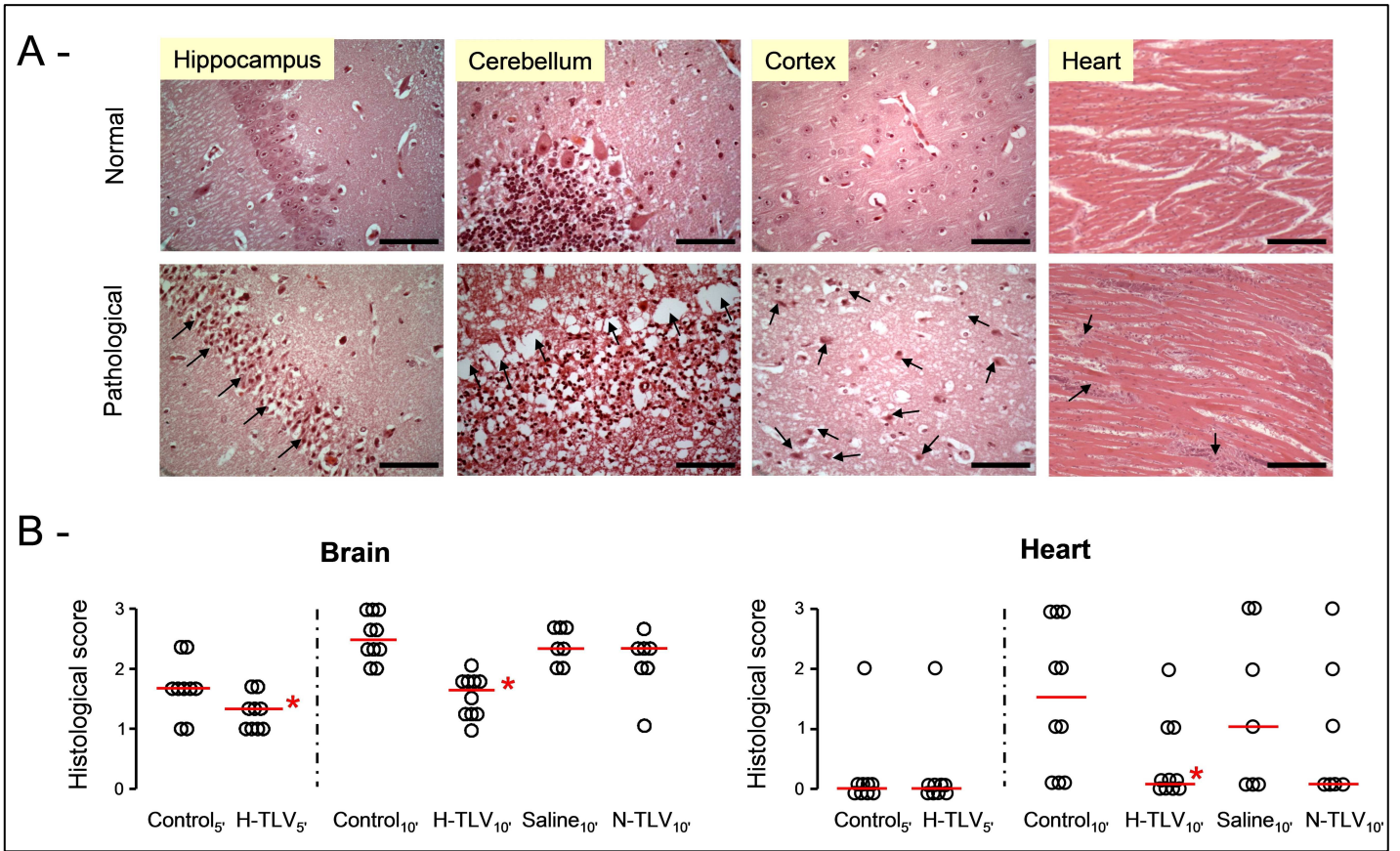


Figure 4

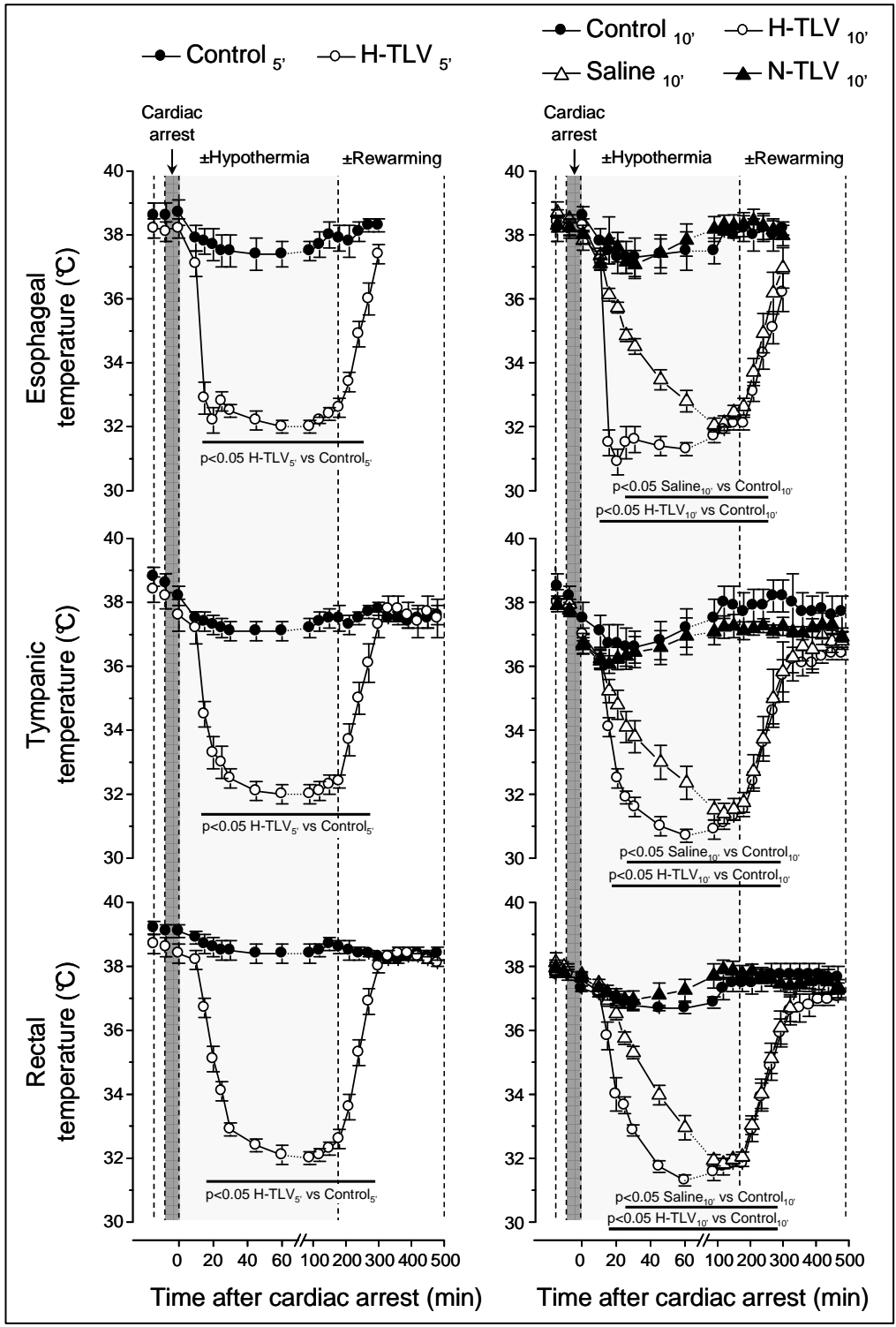


Figure 5

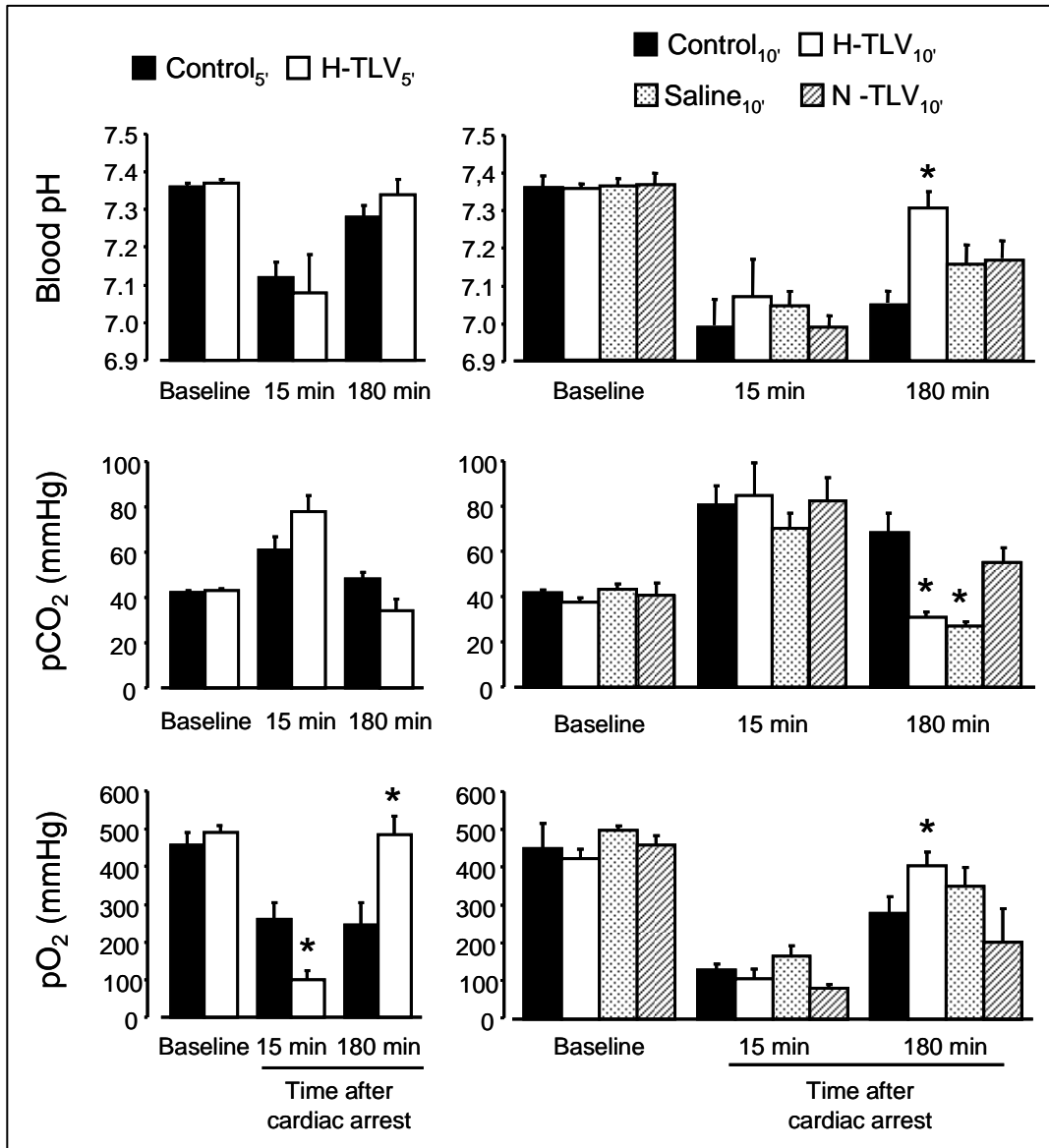
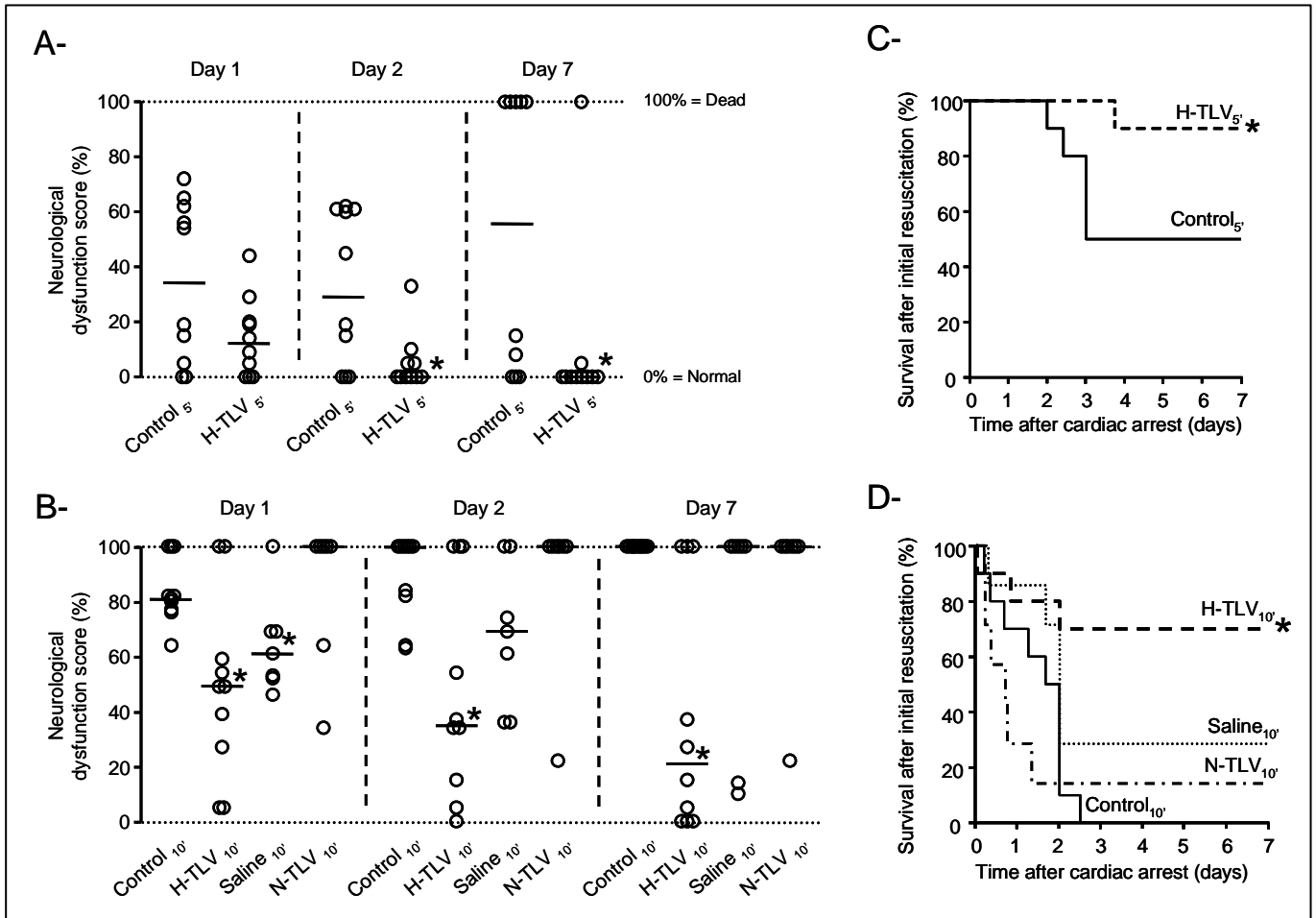


Figure 6



562 **Table 1 :** Groups characteristics during cardiopulmonary resuscitation, including the rate of
 563 successful resuscitation, the time to resumption of spontaneous circulation (ROSC) and the
 564 total amount of epinephrine administered throughout the protocol.

	n	Rate of successfull resuscitation	ROSC (min)	Epinephrine dose ($\mu\text{g}/\text{kg}$)
Control _{5'}	10	10/10	2.4 \pm 0.3	207 \pm 58
H-TLV _{5'}	10	10/10	2.3 \pm 0.3	174 \pm 81
Control _{10'}	15	10/15	4.8 \pm 0.4	684 \pm 118
H-TLV _{10'}	15	10/15	4.2 \pm 0.8	128 \pm 128 *
Saline _{10'}	10	7/10	3.7 \pm 0.7	430 \pm 126
N-TLV _{10'}	10	7/10	3.7 \pm 0.4	509 \pm 64

565 * $p < 0.05$ vs corresponding control value; TLV, total liquid ventilation; H-TLV, hypothermic
 566 TLV; N-TLV, normothermic TLV; Saline, hypothermia induced by intravenous administration
 567 of cold saline combined to external cooling.

568 **Table 2 :** Mean arterial pressure, heart rate, plasma creatinine and alanine aminotransferase
 569 concentrations (ALAT) throughout the experimental protocol in the different groups.

	n	Baseline	Cardiopulmonary resuscitation					Day 1 (n)
			15min	60min	180min	360min	480min	
<u>Epinephrine perfusion</u>		No	Yes	Yes	Yes	Yes	No	
<u>Heart rate (beat/min)</u>								
Control 5'	10	257±11	222±8	221±7	243±11	216±7	220±9	234±8 (10)
H-TLV 5'	10	259±10	202±12	174±6 *	177±9 *	245±9	234±8	244±10 (10)
Control 10'	10	263±10	219±6	220±10	198±8	221±11	231±13	256±17 (7)
H-TLV 10'	10	267±8	167±10 *	158±8 *	167±11	208±12	240±11	252±7 (8)
Saline 10'	7	266±7	200±10	153±7 *	155±13 *	219±10	218±10	226±16 (6)
N-TLV 10'	7	256±13	216±19	207±9	213±12	207±9	221±15	240±28 (2)
<u>Mean arterial pressure (mmHg)</u>								
Control 5'	10	81±3	83±4	82±3	83±1	83±4	80±4	83±4 (10)
H-TLV 5'	10	80±7	81±3	82±5	82±3	83±3	79±3	82±4 (10)
Control 10'	10	80±5	82±3	83±3	81±4	83±2	80±3	79±4 (7)
H-TLV 10'	10	83±4	81±4	82±3	81±3	81±4	80±4	79±6 (8)
Saline 10'	7	80±8	86±6	89±2	78±5	82±5	76±6	83±9 (6)
N-TLV 10'	7	78±7	78±5	78±1	78±4	78±5	75±7	88±4 (2)
<u>Plasma creatinine concentrations (mg/l)</u>								
Control 5'	10	10±1	11±1	-	10±1	-	-	10±1 (10)
H-TLV 5'	10	10±0	12±1	-	11±1	-	-	10±1 (10)
Control 10'	10	9±1	13±1	-	14±2	-	-	11±1 (7)
H-TLV 10'	10	10±0	13±1	-	12±1	-	-	11±1 (8)
Saline 10'	7	9±1	10±1	-	10±1	-	-	10±1 (6)
N-TLV 10'	7	9±1	11±1	-	12±1	-	-	13±6 (2)
<u>Plasma ALAT concentrations (U/l)</u>								
Control 5'	10	29±5	31±4	-	33±4	-	-	35±9 (10)
H-TLV 5'	10	25±3	26±2	-	43±5	-	-	30±6 (10)
Control 10'	10	44±13	79±25	-	115±32	-	-	60±17 (7)
H-TLV 10'	10	48±3	65±5	-	111±27	-	-	83±14 (8)
Saline 10'	7	32±2	48±4	-	101±30	-	-	62±27 (6)
N-TLV 10'	7	31±5	66±10	-	96±13	-	-	94±37 (2)

570 * $p < 0.05$ vs corresponding control value; TLV, total liquid ventilation; H-TLV, hypothermic
571 TLV; N-TLV, normothermic TLV; Saline, hypothermia induced by intravenous administration
572 of cold saline combined to external cooling.