



UNIVERSITY OF
Rhode Island

Evidence for Benefits of Rapid Induction of Hypothermia

William J. Ohley, Ph.D.

June 23, 2008

Evidence for Benefits of Rapid Induction of Hypothermia

William J. Ohley, Ph.D., University of Rhode Island

June 23, 2008

Introduction

There have been a number of animal and clinical trials that have applied hypothermia as a treatment protocol in the setting of post-ischemic injury. A large group of studies have examined the amelioration of complications of sudden collapse of the circulation. The two well known clinical trials were performed in Europe [1] and in Australia [2]. Largely because of these trials, the 2005 AHA guidelines advise cooling patients who have been resuscitated from a VF rhythm yet remain in a comatose state.

Animal Studies

In the above two clinical trials the average time to reach hypothermia was typically well in excess of 120 minutes, even though the treatment was started relatively early. Based on a review of animal research of therapeutic hypothermia as a treatment for brain ischemia, it is probable that faster, earlier cooling would be beneficial. The animal trials have typically used dogs, swine or rats. The typical times to achieve the targeted hypothermic temperature were from 5 to 20 minutes. This depended on the depth (temperature of hypothermia) and on the methods used to achieve hypothermia. Thus, a major difference between many clinical studies and the animal work has been the rate of hypothermia induction. The animal trials have all induced hypothermia relatively quickly, while the clinical studies have been very slow by comparison. Some of the experimental evidence supporting the benefits of early cooling in the treatment of cerebral ischemia is summarized in Table 1 below and in the subsequent discussion:

Table 1:
Experimental Evidence in Cerebral Ischemia Supporting the Positive Effects of Early Cooling
[3]

REFERENCE	PATTERN OF INJURY	START OF COOLING	RESULT
Busto et al 1989 [4]	2-vessel occlusion Rats	After Reperfusion 5 vs. 30 min, 34°C	Protection with early cooling only
Carroll et al 1992 [5]	Gerbils	Before CPR, after reperfusion 0 vs. 3 h	Before CPR far more effective than after ROSC; no protection with late cooling
Kuboyama et al 1993 [6]	Cardiac arrest Dogs (n=22)	After ROSC 0 vs. 15 min, 34°C	Higher degree of protection after early cooling
Coimbra et al 1994 [7]	2-vessel occlusion Rats	After reperfusion 2, 6, 12, 24 vs. 36h	Protection with cooling starting at 2, 6, and 12 h only
Abella et al 2004 [8]	Cardiac arrest Mice	During vs. after cardiac arrest	Better survival with early cooling
Takata et al 2005 [9]	Cardiac arrest Rats (n=42)	After CPR 0, 5, 10 vs. 20 min, 31°C	Glutamate is reduced with early cooling only

While the rate of hypothermia induction has not been studied per se in animal work, there have been a number of experimental variables examined. Reasons for this situation are most likely due to the difficulty of experimentally controlling the rate of temperature fall. Thus, the most significant variables tested thus far are the time of hypothermia onset and the absolute target temperature. In addition, the length of time hypothermia is maintained has also been studied in the brain ischemia setting, as well as the rate of re-warming.

Finally there are animal studies which examine the biochemistry associated with hypothermia in ischemia. The major hypotheses examined are the temperature dependence of the reactions.

As an example, [Figure 1](#) below shows a temperature vs. time graph from Nozari et al [10], using a series of dogs with induced cardiac arrest. The animals were held in a state of VF for 60 minutes with hypothermia started early, after 10 minutes of VF, and a third group in which hypothermia induction was delayed for an additional 10 minutes. The resulting differences between the early and later hypothermia application in this model are shown in the table at the bottom of [Figure 1](#).

Figure 1: Effects of early vs late cooling in ventricular fibrillation (Nozari et al, 2006)

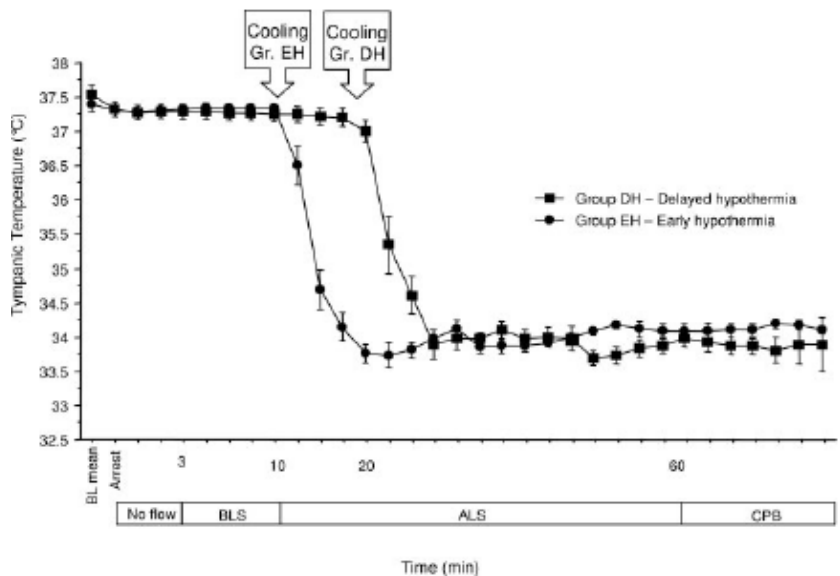


Figure 2. Tty during CA. Gr indicates group.

	Delayed hypothermia	Early hypothermia
OPC 5 or death	●●●●●●●●	●
OPC 4		●
OPC 3		●
OPC 2		●
OPC 1	●	●●●●
NDS (%)	[0]	5.5 (0-57)
HDS	[32, 38, 45]	0 (0-96)
MDS (%)	68.5 (47-93)	58.5 (43-93)

Figure 5. Final 96-hour outcome. Each dot represents a dog. Values are expressed as median (range). Values in brackets represent HDS at 4 to 37 hours of reperfusion. MDS indicates gross myocardial damage score.

It was concluded that in this model, the timing of hypothermia is crucial. From this data it can also be inferred that achieving a therapeutic level quickly is quite important; outcomes in the study were significantly worsened by a ten minute delay in reaching the target temperature.

Other studies have looked at the temperature level of hypothermia. Kollmar et al [11] demonstrated, in a experimental stroke model using rats, that of a temperature range of 32 to 37° C, the best results using neuro score, edema, infarct size, and invasion of leukocytes was at 34°C. Thus in this setting of middle cerebral artery occlusion, 34°C provided the best result. In this study it is further noted that hypothermia was induced over an approximately 20 minute period, where the lower temperatures required more time.

Busto et al [4] performed experiments with delayed application of hypothermia in rats with induced cerebral ischemia. Early application within 5 minutes of recirculation provided viable neurons in the CA1 region of the hippocampus while in the 30 minute late group this sector of the brain was severely damaged.

Carroll et al [5] reported in a gerbil model that (1) hypothermia during ischemia protects the brain from damage; 2) Hypothermia initiated immediately following reperfusion must have a duration of 2 hours or more to be effective and 3) Six hours of hypothermia is effective if initiated within 1 hour of reperfusion. The difference in neuro-protection between a one hour delay was 49% percent vs. 77% with no delay. These results are shown in [Figure 2](#) below:

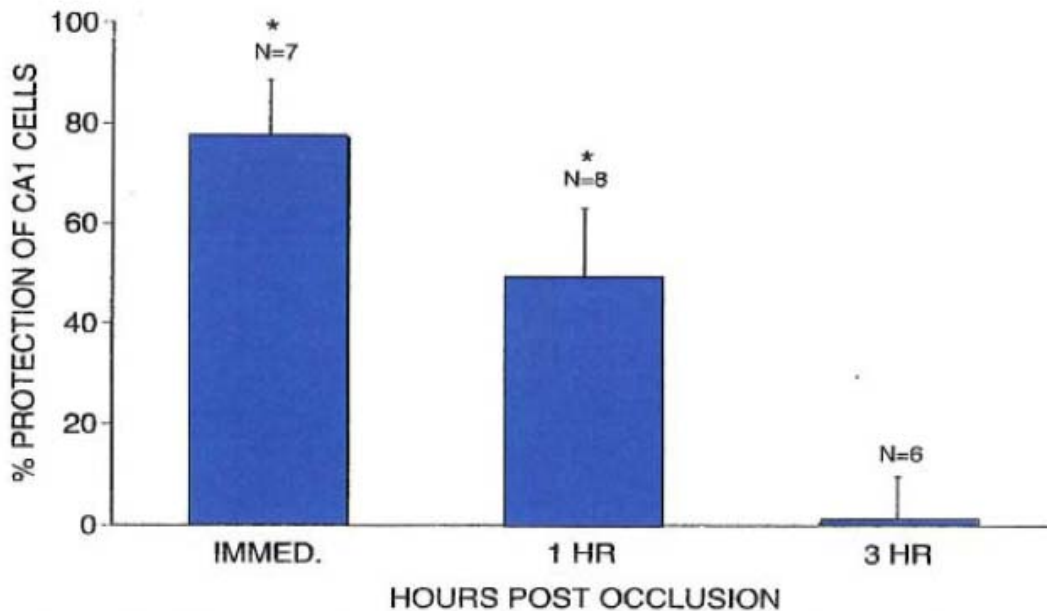


Fig. 2. Gerbils were subjected to 5 minutes of normothermic ischemia. Six hours of hypothermia were initiated at the times indicated following reperfusion. Values are mean \pm SEM. * indicates values significantly different from control.

Carroll et al. Metab Brain Dis, 1992

Kawamura et al [12] demonstrated in an ischemic- reperfusion model in the rat that hypothermia begun immediately and at 1 hour post reperfusion did provide neuro-protection but not if begun at 3 and 4 hours after reperfusion. They concluded that prolonged delay worsened the injury.

Froehler et al [13] provide a review of hypothermia mechanisms in the setting of neuro-protection following cardiac arrest. There are two distinct time points where cell death is caused:

at the initial ischemia, and upon reperfusion. There are different modes of cell death in each of these cases. In the case of ischemia, cellular necrosis occurs and results in cell death by membrane breakdown. The delayed neuronal cell death that occurs during reperfusion can be categorized as either apoptosis or autophagocytosis. ATP is typically depleted in ischemia within 4 minutes. This leads to ion pump failure, and subsequently to glutamate release and lipolysis. The lipolysis that occurs causes an accumulation of free fatty acids such as arachidonate. Early application of hypothermia can slow down this set of reactions.

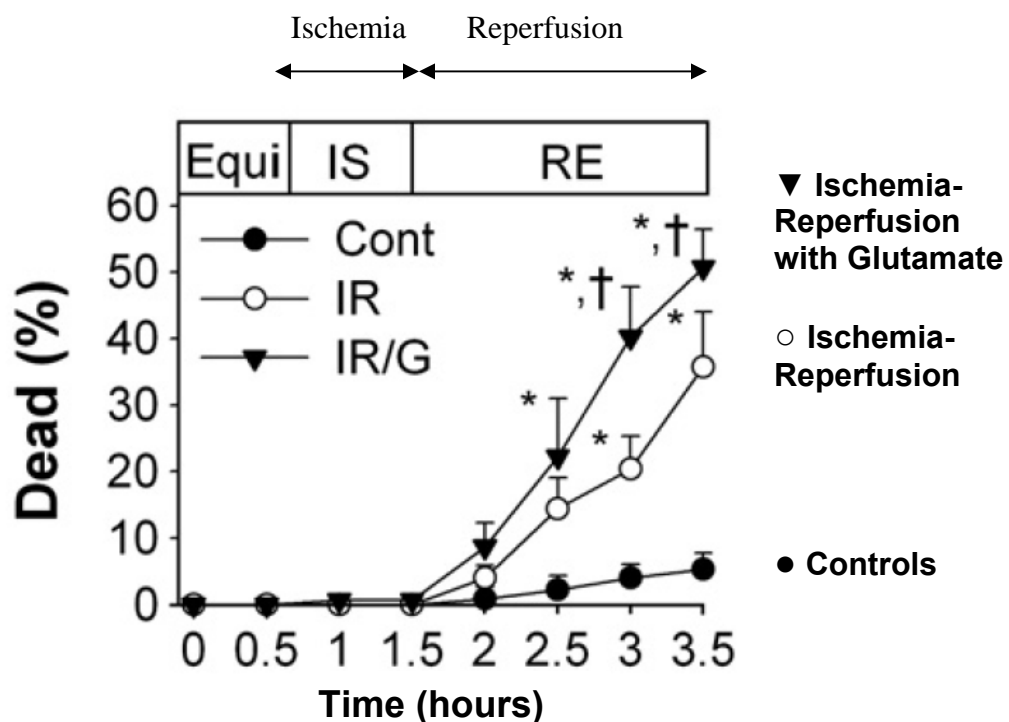
During reperfusion, rapid metabolism of the excess arachidonate takes place and creates oxygen radicals. These free radicals then peroxidize the phospholipids, especially in the cell membrane. This results in structural damage to the cell membrane. This action begins within 15 minutes of reperfusion and can continue for up to 78 hours. Thus later applications of hypothermia can also be beneficial to some extent.

Ischemia also yields inflammation. This is characterized by the appearance of microglia within 2 to 4 hours. The post ischemic inflammation generally results in net tissue damage. Again the later application of hypothermia will have an effect on these reactions. However earlier application will minimize the production of by products.

Hypothermia is able to affect many of these pathways because the reactions are temperature dependant. The colder the cells are, the slower the reactions. These mechanisms also argue for early and rapid initiation of hypothermia. The sooner that therapeutic temperatures are reached, the fewer adverse reactions will occur.

A recent study by a group at University of Chicago [14] showed that neurons tolerated as much as three hours of normothermic ischemia, but cell death progressed very rapidly in the hours immediately following reperfusion (see [Figure 3](#) below):

Figure 3: Neuronal Cell Death vs. Time (1-hour ischemia/reperfusion model) [14]



Other studies such as those previously cited have shown that hypothermia reduces glutamate release and cell injury during this reperfusion phase. From this it is apparent that **every minute that hypothermia therapy is delayed increases the risk of permanent neurological injury.**

A new study of post-resuscitation hypothermia, published in Critical Care Medicine in March 2008 [15], sheds additional light on this subject. This study was conducted by Dr. Fritz Sterz and his Experimental Resuscitation Research Group of the University of Vienna. This was the same group that conducted the large clinical trial that led to the 2005 AHA guidelines for post-resuscitation hypothermia. In this study tests were conducted using a 30Kg post-resuscitation swine model. All animals were subjected to 10 minutes of untreated ventricular fibrillation cardiac arrest, followed by 8 minutes of CPR and drugs, and then attempted defibrillation. The 16 animals surviving this sequence were randomized as follows:

Control Group: Animals were maintained at normothermia.

Study Group: Animals were cooled with the TSS (the LRS ThermoSuit System, an approach that creates the conditions of ice water immersion), maintained hypothermic for 14 hours, and then gradually rewarmed.

Neurological and physical scoring was conducted over the next 9 days for all animals by an examiner who was blinded to the treatment assignments. Blood samples were taken periodically during this time to measure markers of brain recovery. After the 9 day recovery period the animals were sacrificed and brain tissue samples were harvested for histological examination.

In this study, the TSS produced rapid cooling rates of approximately 0.4 C°/minute (see [Figure 4](#) below).

Figure 4: Temperature Curves for Post Arrest Animal Study of LRS ThermoSuit (Janata et al, Critical Care Medicine 2008 [15])

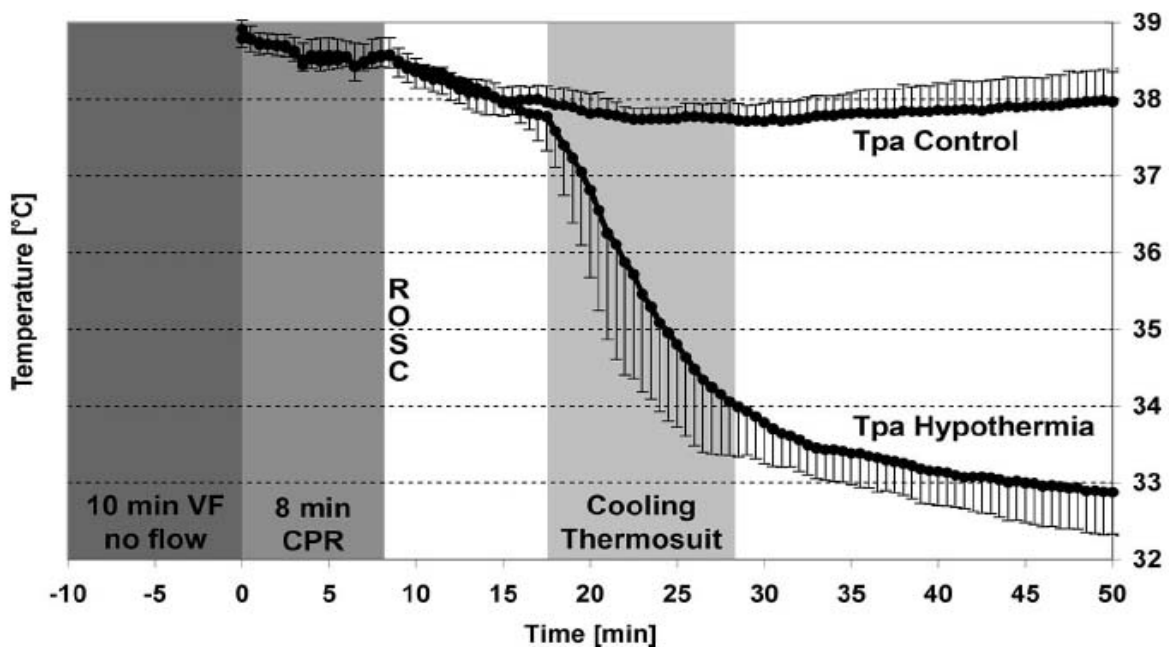
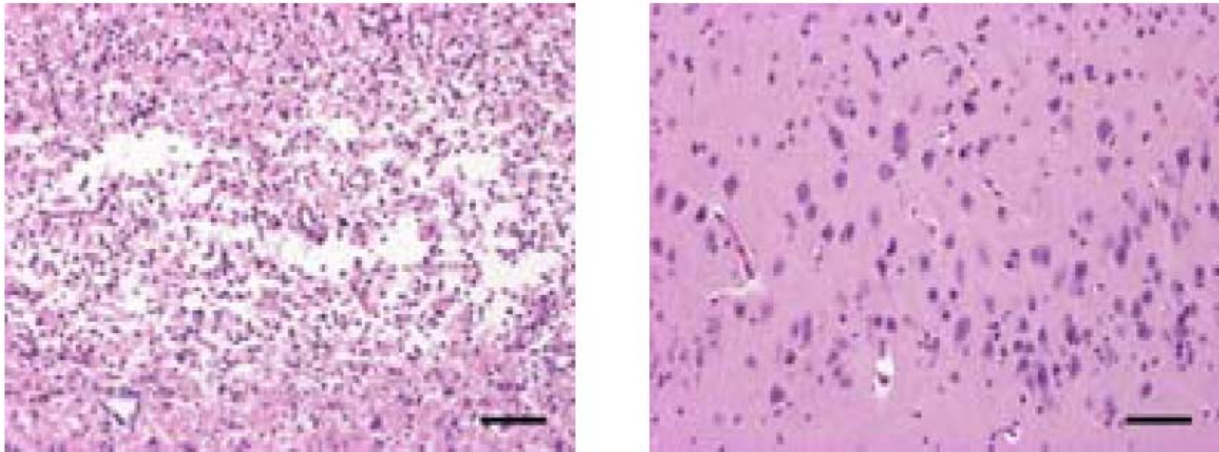


Figure 2. Experimental protocol and pulmonary artery temperature (*Tpa*, mean and SD) during cardiac arrest, resuscitation, and cooling. *VF*, ventricular fibrillation; *CPR*, cardiopulmonary resuscitation; *ROSC*, return of spontaneous circulation.

Eight of the eight animals cooled with the TSS under this protocol showed excellent neurological and physical recovery after nine days and were free of adverse effects. In comparison, seven of the eight normothermic control animals exhibited significant neurologic injury after nine days.

The blinded histological analysis of brain tissue showed that the animals cooled with the ThermoSuit had a significantly lower level of brain cell injury than did those animals that did not receive cooling (see [Figure 5](#) below).

Figure 5: Left: Swine brain cells with extensive necrosis after cardiac arrest followed by normothermic recovery; **Right:** Normal swine brain cells after cardiac arrest followed by rapid cooling to 33°C induced by LRS ThermoSuit [16].



The summary of outcomes from this study is outlined in [Figure 6](#) below.

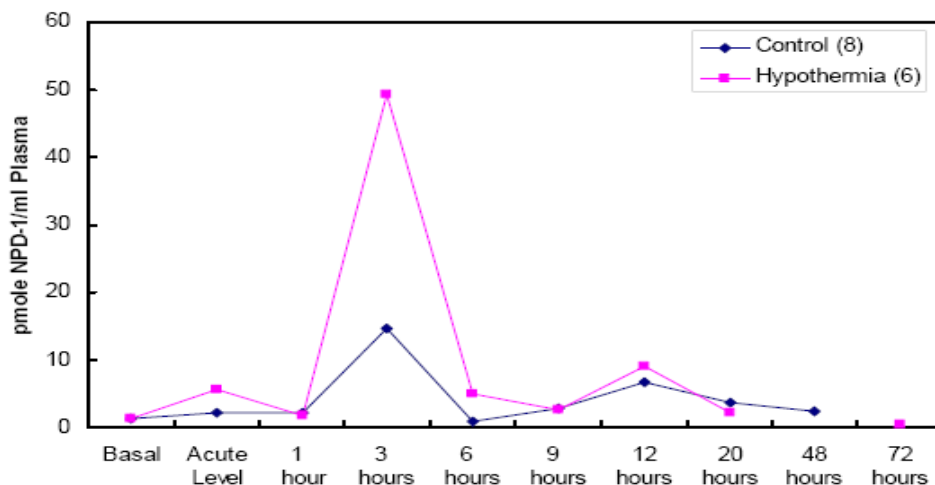
Figure 6: Outcomes in Swine Following 10 Minutes Cardiac Arrest and 8 minutes CPR* [15]

Outcome	Hypothermia	Control
OPC 1	••••••••	•
OPC 2		•••
OPC 3		••••
OPC 4		
OPC 5		
NDS	0 (0; 4)%	39 (19; 55)%
HDS	71 (61; 84)	132 (124; 174)

*Outcomes in terms of final overall performance categories (*OPC 1-5*) on day 9. Each dot represents one swine. Neurologic deficit scores (*NDS*) and histologic damage scores (*HDS*) are given as median (interquartile range). *Hypothermia*, hypothermia group (n = 8); *Control*, control group (n = 8); *ROSC*, restoration of spontaneous circulation. *OPC* at 9 days, $p = .002$; *NDS* at 9 days, $p = .001$; *HDS* at 9 days, $p < .001$.

The blood samples collected from the animals in this study were analyzed for the presence of neuroprotective compounds at various time points during recovery [17]. The results demonstrated that the rapid cooling provided by the TSS was associated with a **five-fold increase** in Neuroprotectin D-1 (NPD-1), an important chemical released by the body to help the brain recover from a sustained lack of oxygen [18] (see Figure 7 below).

Figure 7: Augmentation of Neuroprotectin D-1 following cardiac arrest and resuscitation in the swine model. The primary release of NPD-1 was three hours after resuscitation. Rapid hypothermia therapy delivered with the TSS increased NPD-1 release by a factor of five [17].



The timing of the NPD-1 release clearly showed that **cooling more than three hours after resuscitation misses the window for maximal augmentation of NPD-1 release**. This provides important new evidence in support of the need for early cooling following cardiac arrest. The researchers in the above studies concluded that the LRS ThermoSuit was safe and effective in inducing therapeutic hypothermia in pigs after cardiac arrest. Neurological performance scores after prolonged cardiac arrest were improved significantly in animals cooled with the TSS as compared to control animals.

Human Studies

A number of peer-reviewed published clinical studies of post-resuscitative hypothermia further support the benefits of rapid cooling in post-ischemic conditions. For example, the 273-patient prospectively randomized study by Holzer et al [1] cooled comatose post-arrest patients to 32 to 34°C within an average of 8 hours after resuscitation. 39% of normothermic control patients had a favorable neurologic outcome at six months, while 55% of cooled patients had a favorable outcome. This reflected a 41% improvement in favorable outcome at six months. In the study of 77 patients by Bernard et al [2], patients were cooled more quickly (2.5 hours from resuscitation to 33.5 °C) by starting the cooling process in the ambulance. In this study, 26% of normothermic controls had a favorable outcomes, while 49% of cooled patients had favorable outcomes. This corresponded to an 88% increase in favorable outcome. These results are summarized in Table 2 below:

Table 2: Outcome Improvements Slow and Rapid Cooling in Comatose Post-Arrest Patients

STUDY	NUMBER OF PATIENTS	TIME FROM RESUSCITATION TO HYPOTHERMIA	% INCREASE IN PATIENTS WITH FAVORABLE OUTCOMES
[1]	273	8 hours	41%
[2]	77	2.5 hours	88%

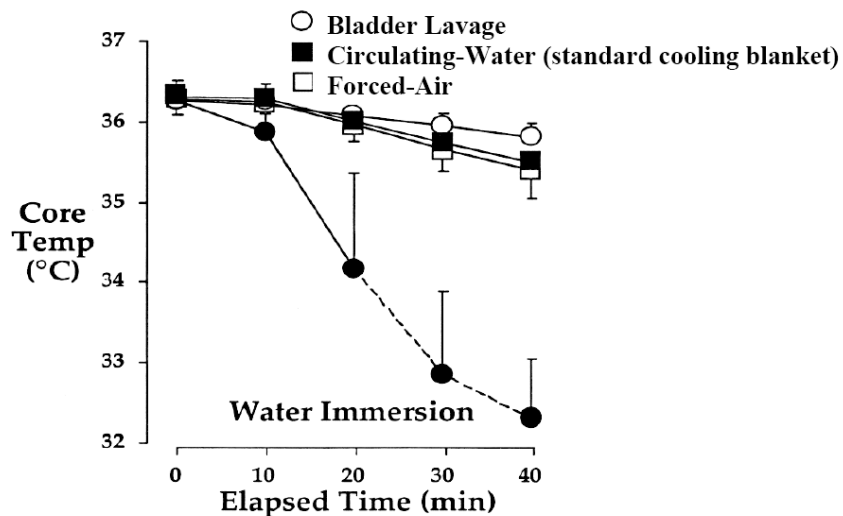
The above comparison and previously cited animal data support the hypothesis that rapid early cooling may improve outcomes in post-arrest patients, compared to slower, later cooling.

The relatively early cooling achieved in the above study by Bernard et al required that ice packs be applied to patients in the pre-hospital setting for early initiation of cooling. This approach presents some drawbacks (the ambulance crews need to maintain the ice packs in constant readiness, and cooling procedures may divert field caregivers from the tasks of patient stabilization and transport). Ice packs also introduce a risk of localized freezing of tissues (frostbite), and 30 minutes is the maximum safe period of application [19, 20]. Bernard and others have also used intravenous cold saline to induce hypothermia in pre-hospital patients [eg. 21, 22, 23], but this approach is limited in its cooling capability; furthermore, patients tend to passively rewarm following its discontinuation [22].

Because of the challenges related to implementation of available pre-hospital cooling methods, the concept of using a rapid in-hospital cooling method is attractive. Invasive cooling catheters have been developed for this purpose, with reported cooling rates on the order of 1.1 C°/hr with the Alsius cooling catheter [25]. However, these introduce an additional delay associated with the insertion procedure, and have been reported to be associated with risks such as deep venous thrombosis [24] and an increased rate of arrhythmias in comparison with surface cooling methods [25]. Special cooling blankets with thermally conductive gel coatings have been introduced, but these typically require hours to cool the patient to the therapeutic level (reported cooling rate of 1.2 C°/hr with the Medivance Arctic Sun device [26]).

The ice water immersion approach used in the ThermoSuit animal study previously cited [15], has been investigated in humans, and is known to be highly effective [27, 28]. In a study of human volunteers [27], it was demonstrated that ice water immersion provided significantly faster cooling rates than those achieved with other methods evaluated. As shown in Figure 8 below, ice water immersion enabled cooling of the test subjects from normothermia to 34°C in approximately 20 minutes (approximately 6.6 C°/hr cooling rate). This cooling was significantly faster than forced cool air, conventional cooling blankets (circulating water-filled), and circulation of cold fluid into the urinary bladder, each of which only cooled a fraction of a degree in 40 minutes (approximately 1.2 C°/hr cooling rate).

Figure 8: Comparison of Ice Water Immersion with Bladder Lavage, Standard Cooling Blanket, and Forced-Air Cooling Methods in Humans [19].



Given the above published cooling rates for various cooling methods, the time to cool patients from a normothermic condition of 37°C to a 34°C target for therapeutic hypothermia would be as follows:

Table 3: Comparison of Cooling Rates and Cooling Times for Various Methods

COOLING METHOD	PUBLISHED COOLING RATE (C°/HR)	TIME TO COOL PATIENTS FROM 37°C TO 34°C
Conventional Cooling Blanket [27]	1.2	150 minutes
Medivance Arctic Sun Gel-Coated Cooling Blanket [26]	1.2	150 minutes
Alsius Cooling Catheter [25]	1.1	163 minutes
Ice Water Immersion [27]	6.6	27 minutes

Early clinical reports of the LRS ThermoSuit device have reported cooling rates similar to those of the above ice water immersion studies. In the initial series of patients treated, an average cooling treatment of only 36 minutes was required to induce hypothermia [29]. It is likely that the addition of mild vasodilators such as magnesium sulfate will make this cooling method even faster [30].

The rapid cooling associated with ice water immersion raises hypothetical questions related to safety. However, in the initial series of patients cooled with the LRS ThermoSuit System, there were no adverse events associated with the cooling process [29]. Even more rapid cooling has long been provided during surgical cardiopulmonary bypass procedures, and this has not been reported to be associated with patient injury. The typical cooling rate during a cardiopulmonary bypass procedure is approximately 40 C°/hr [36]. This cooling rate is more than five times as rapid as that provided by ice water immersion cooling.

Other studies have investigated the effect of therapeutic hypothermia on treatment of acute myocardial infarction [31, 32] and traumatic brain injury [33, 34, 35]. In those conditions as well, there is much evidence in support of the need for cooling to be provided early for it to be most effective.

Conclusions

- **Therapeutic hypothermia offers potential benefit for the treatment of cerebral ischemic conditions including cardiac arrest and stroke, as well as acute myocardial infarction and traumatic brain injury.**
- **There is much evidence to support the assertion that the earlier and more quickly cooling is provided, the more effective it will be in improving outcomes.**
- **In the treatment of cerebral ischemic conditions, rapid cooling methods such as the ice water immersion approach should be considered to minimize the time to target temperature.**

Selected References

1. Holzer M et al, "Mild Therapeutic Hypothermia to Improve the Neurologic Outcome after Cardiac Arrest", *N Engl J Med* 2002, Vol. 346, No. 8, 549-556.
2. Bernard SA et al, "Treatment of Comatose Survivors of Out-of-Hospital Cardiac Arrest with Induced Hypothermia", *N Engl J Med* 2002, Vol. 346, No. 8, 557-563.
3. Bottinger B, "Underlying mechanisms: How Hypothermia Can Protect the Brain", *Hypothermia Workshop, International Scientific Hypothermia Group, Oct. 31, 2006*.
4. Busto R, Dietrich WD, Globus MY, Ginsberg MD, "Postischemic moderate hypothermia inhibits CA1 hippocampal ischemic neuronal injury", *Neurosci Lett*. 1989 Jul 3; 101(3): 299-304.
5. Carroll M, Beek O, "Protection against hippocampal CA1 cell loss by post-ischemic hypothermia is dependent on delay of initiation and duration", *Metab Brain Dis*. 1992 Mar;7(1):45-50.
6. Kuboyama K, Safar P, Radovsky A, Tisherman SA, Stezoski SW, Alexander H, "Delay in cooling negates the beneficial effect of mild resuscitative cerebral hypothermia after cardiac arrest in dogs: a prospective, randomized study", *Crit Care Med*. 1993 Sep;21(9):1265-6.
7. Coimbra C, Wieloch T, "Moderate hypothermia mitigates neuronal damage in the rat brain when initiated several hours following transient cerebral ischemia", *Acta Neuropathologica* 1994; 87 (4), 325-331.
8. Abella B.S., "Intra-Arrest Cooling Improves Outcomes in a Murine Cardiac Arrest Model et al", *Circulation*, 6/8/2004, pp. 2786-91.
9. Takata K, Takeda Y, Sato T, Nakatsuka H, Yokoyama M, Morita K, "Effects of hypothermia for a short period on histologic outcome and extracellular glutamate concentration during and after cardiac arrest in rats", *Crit Care Med*. 2005 Jun;33(6):1449-52.
10. Nozari et al "Critical Time Window for Intra-Arrest Cooling With Cold Saline Flush in a Dog Model of Cardiopulmonary Resuscitation", *Circulation*. 2006;113:2690-2696.
11. Kollmar R et al, "Different Degrees of Hypothermia After Experimental Stroke", *Stroke* 2007; 38: 1585-1589.
12. Kawamura N, Schmelzer JD, Wang Y, Scheichel AM, Low PA, "The therapeutic window of hypothermic neuroprotection in experimental ischemic neuropathy: protection in ischemic phase and potential deterioration in later reperfusion phase", *Exp Neurol*. 2005 Oct;195(2):305-12.
13. Froehler MT, Geocadin RG, "Hypothermia for neuroprotection after cardiac arrest: Mechanisms, clinical trials and patient care", *J Neurol Sci*. 2007 Oct 15;261(1-2):118-26.
14. Li D et al, "Reperfusion accelerates acute neuronal death induced by simulated ischemia", *Experimental Neurology* 206 (2007) 280-287.
15. Janata A, Weihs W, Bayegan K, Schratter A, Holzer M, Behringer W, Schock RB, Losert UM, Springler G, Schmidt P, Sterz F, "Therapeutic hypothermia with a novel surface cooling device improves neurologic outcome after prolonged cardiac arrest in swine", *Critical Care Medicine*: Volume 36(3) March 2008, pp 895-902.
16. Springler G, Janata A, Weihs W, Bayegan K, Schratter A, Behringer W, Schock RB, Freedman RJ, Losert UM, Laggner AN, Schmidt P, Sterz F "Reduction of neuronal Damage after prolonged cardiac arrest with mild therapeutic hypothermia",

- Resuscitation Science Symposium, American Heart Association Scientific Sessions, 2007.*
17. White C, Marcheselli VL, Janata A, Schratte A, Weihs W, Bayegan K, Milani RV, Bazan NG, "Lipid Mediators as Novel Biomarkers and Surrogate Indicators of Neurologic Recovery after Cardiac Arrest in a Hypothermic Swine Model", *AHA RESS (2006), Circulation Supplement II* Vol 114 no 18.
 18. Bazan NG, "Searching for a New Strategy to Protect the Brain", *Cerebrum – The Dana Forum on Brain Science*, Jan. 2006, pp. 12-20.
 19. Graham C, Stevenson J, "Frozen chips: an unusual cause of severe frostbite injury", *Br J Sports Med* 2000;34:382-384.
 20. Kerr KM, Daley L, Booth L, et al, *Guidelines for the management of soft tissue (musculoskeletal) injury with Protection, Rest, Ice, Compression and Elevation (PRICE) during the first 72 hours*. London: Chartered Society of Physiotherapy, 1999.
 21. Bernard S. et al, "Induced hypothermia using large volume, ice-cold intravenous fluid in comatose survivors of out-of-hospital cardiac arrest: a preliminary report", *Resuscitation* 2003, 56, 9-13.
 22. Kliegel A et al, "Intravenous Administration of Cold Fluids, Sedation, Analgesia and Muscle Relaxation if Sufficient for Induction but Not for Maintenance of Therapeutic Hypothermia after Cardiac Arrest", ERC 2006 Proceedings, *Resuscitation* April 2006, p. 58.
 23. Polderman KH et al, "Induction of hypothermia in patients with various types of neurologic injury with use of large volumes of ice-cold intravenous fluid", *Crit Care Med* 2005 Dec;33(12):2744-51.
 24. Simosa HF et al, "Increased risk of deep venous thrombosis with endovascular cooling in patients with traumatic head injury", *Am Surg* 2007 May;73(5):461-4.
 25. Arrich J et al, "Clinical application of mild therapeutic hypothermia after cardiac arrest", *Crit Care Med* 2007 Vol. 35, No. 4, pp. 1041-1047.
 26. Haugk M et al, "Feasibility and efficacy of a new non-invasive surface cooling device in post-resuscitation intensive care medicine", *Resuscitation* 2007 75, pp. 76-81.
 27. Plattner, Olga MD; Kurz, Andrea MD; Sessler, Daniel I. MD; Ikeda, Takehiko MD; Christensen, Richard BS; Marder, Danielle BS; Clough, David MD,, " Efficacy of Intraoperative Cooling Methods", *Anesthesiology*. 87(5):1089-1095, November 1997.
 28. C. I. Proulx, M. B. Ducharme, and G. P. Kenny, "Effect of water temperature on cooling efficiency during hyperthermia in humans", *J Appl Physiol* 94(4):1317-1323, 2003.
 29. Holzer M., Janata A, Haugk M, Krizanac D, Sterz F, "Efficacy and Safety of a Novel Rapid Non-invasive Surface Cooling Device for Induction of Therapeutic Hypothermia in Patients after Cardiac Arrest", *Resuscitation Science Symposium, American Heart Association Scientific Sessions, 2007.*
 30. Zweifler Rm, Voorhees ME, Mahmood MA, Parnell M, "Magnesium sulfate increases the rate of hypothermia via surface cooling and improves comfort", *Stroke*. 2004, Oct;35(10):2331-4.
 31. Dae MW et al, "Effect of endovascular cooling on myocardial temperature, infarct size, and cardiac output in human-sized pigs", *Am J Physiol* 2002; 282: H1584-H1591.
 32. O'Neill WW, "Acute Myocardial Infarction: Current Strategies, Future Approaches", *Transcatheter Cardiovascular Therapeutics Meeting*, Sept. 2003.

33. Shiozaki T et al, "Selection of severely head injured patients for mild hypothermia therapy", *Journal of Neurosurgery* 1998; 89 (2) 206-11.
34. Clifton GL, Miller ER, Choi SC, Levin HS, McCauley S, Smith KR Jr, Muizelaar JP, Wagner FC Jr, Marion DW, Luerssen TG, Chesnut RM, Schwartz M., "Lack of effect of induction of hypothermia after acute brain injury", *N Engl J Med.* 2001 Feb 22;344(8):556-63.
35. Clifton GL. "Is keeping cool still hot? An update on hypothermia in brain injury", *Current Opinion in Critical Care.* 2004 Apr;10(2):116-9.
36. Stone J et al, "Do Standard Monitoring Sites Reflect True Brain Temperature When Profound Hypothermia Is Rapidly Induced and Reversed?", *Anesthesiology: Vol.* 82(2) Feb. 1995, pp. 344-351.