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Evidence for Benefits of Rapid Induction of Hypothermia

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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, 34ºC	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, 34ºC	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, 31ºC	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)

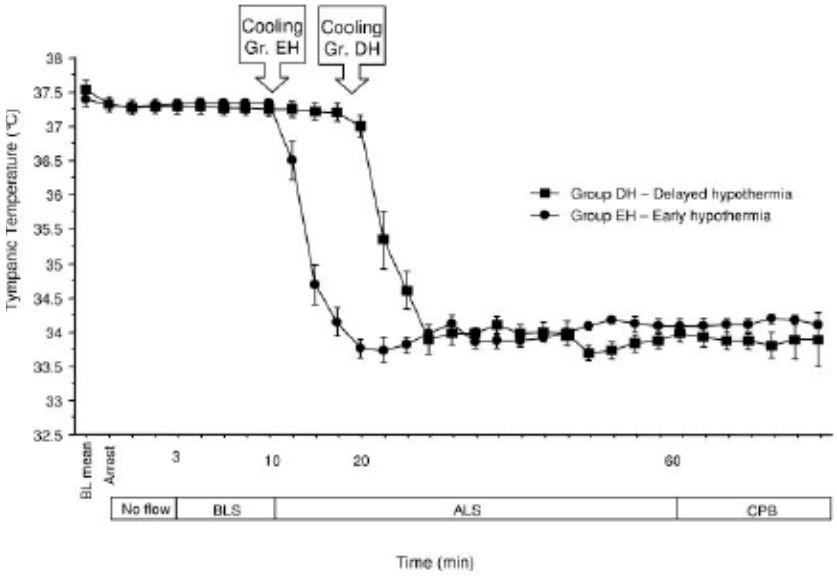


Figure 2. Tty during CA. Gr indicates group.

	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 (0-98)
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 37º C, the best results using neuro score, edema, infarct size, and invasion of leukocytes was at 34ºC. Thus in this setting of middle cerebral artery occlusion, 34ºC 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:

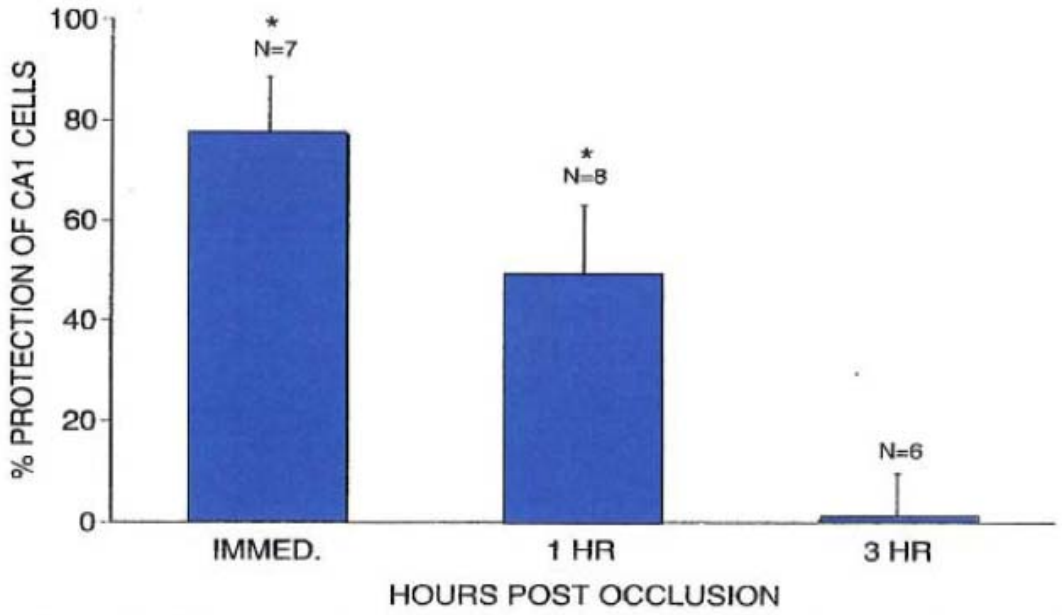


Fig. 2. Gerbils were subjected to 5 minutes of normothermic ischemia. Six hours of hypothermia were initiated at the times indicated following reperfusion. Values are mean ± SEM. * indicates values significantly different from control.

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.

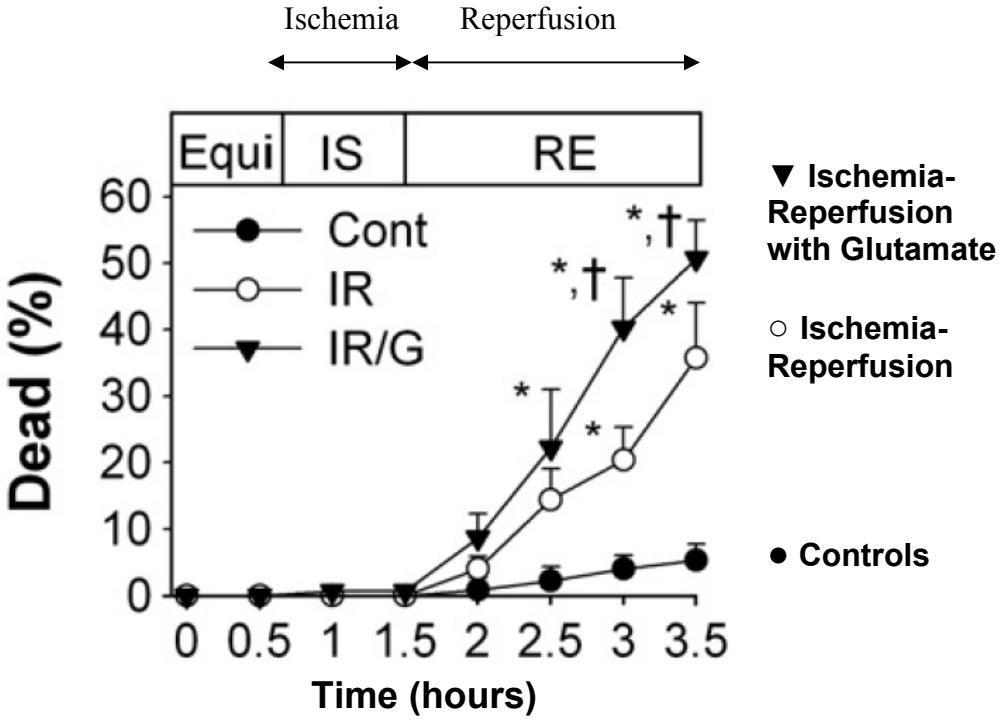
During reperfusion, rapid metabolism of the excess arachidonate takes place and creates oxygen radicals. These free radicals then perioxidize the phospholipids, especially in the cell membrane. This results in structural damage to the cell membrane. This action begins with in 15 minutes of reperfusion 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 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])

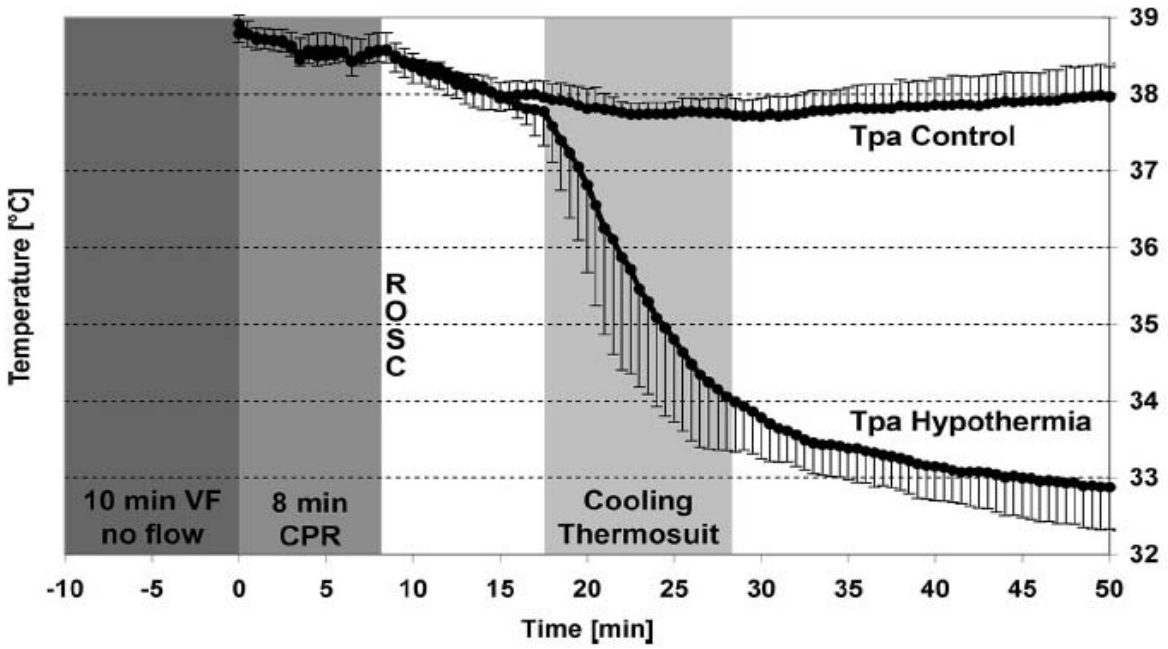
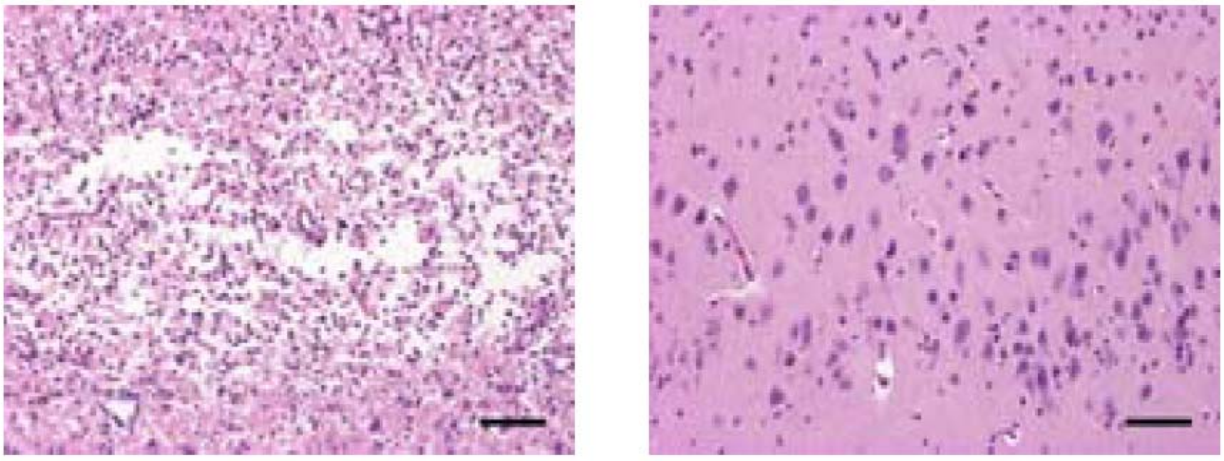


Figure 2. Experimental protocol and pulmonary artery temperature (*Tpa*, mean and SD) during cardiac arrest, resuscitation, and cooling. *VF*, ventricular fibrillation; *CPR*, cardiopulmonary resuscitation; *ROSC*, return of spontaneous circulation.

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 33ºC induced by LRS ThermoSuit [16].



The summary of outcomes from this study is outlined in Figure 6 below.

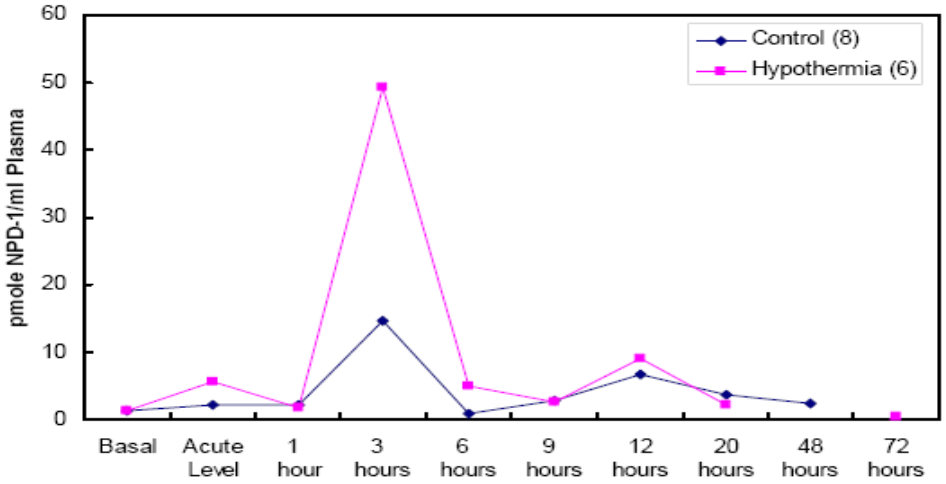
Figure 6: Outcomes in Swine Following 10 Minutes Cardiac Arrest and 8 minutes CPR* [15]

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 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 34ºC 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 ºC) 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 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]. Nevertheless, these results pointed to the promise of earlier cooling

Since the above studies were published, a number of subsequent clinical reports have provided additional support for speeding the delivery of therapeutic hypothermia to comatose post-cardiac arrest patients (see Table 3 below).

Table 3: Clinical Studies Reporting Impact of Timing of Therapeutic Hypothermia

Reference	Results Support Faster Cooling?	Key Results
Wolff B et al, *2009.*	Yes*	"...any hour delay till the coldest T or the target T tended to worsen the likelihood for a favourable outcome by approximately 27% or 31% respectively"
Mooney M, *2009.*	Yes*	"The relative risk of death is 29% higher each hour of delay to first cooling"
Castrén M et al, *2009.*	Yes*	4.2 hrs to target temp: 21.4% recovery 2.1 hrs to target temp: 36.7% recovery
Heard KJ et al, *2010*.	Yes	4.1 hr median cooling time: 38% recovery 3.2 hr median cooling time: 47% recovery
Peberdy MA et al, *2009.*	Yes	6.6 hrs to target temp): 24% recovery 4.1 hrs to target temp): 32% recovery
Bernard S*, 2008.*	No	*Cold IV saline associated with trend for worsened outcomes*
Nielsen N et al, *2009*.	No	5.8 hrs from ROSC to target temp: 44% recovery *80% treated with cold IV Saline; may have masked benefits of earlier cooling)*

1. "Recovery" defined as survival with favorable neurological outcome (CPC-1 or 2)
* Study demonstrated statistically significant benefit of earlier cooling

Wolff et al [38] performed a meta-analysis of results from 49 post-cardiac arrest patients treated in Germany with cooling catheters, and concluded that any hour delay till coldest temperature or target temperature tended to worsen the likelihood for a favorable outcome by approximately 27% ($p = .013$) or 31% ($p = .037$) respectively. The authors concluded, "According to our data, early achievement of MTH (mild therapeutic hypothermia) is a determinant of the final neurologic outcome. Thus, measures to speed up the initiation of cooling therapy after CA appear warranted."

Mooney [39] examined the relationship between timing of cooling and outcomes in 121 post-cardiac arrest patients cooled in Minnesota primarily with surface cooling methods and noted, "The relative risk of death is 29% higher each hour of delay to first cooling" ($p=0.0233$). A subsequent analysis by Mooney of a larger cohort of patients [40] determined that a one hour earlier initiation of cooling was associated with a 25% increase in the probability of patient survival.

Heard et al [42] conducted a prospective randomized trial of 65 post-cardiac arrest patients in 8 U.S. hospitals comparing the use of conventional cooling blankets and ice with the Medivance Arctic Sun device (an adhesive-faced cooling blanket). The Arctic Sun cooled patients to target temperature in a median of 3.2 hours, while cooling blankets and ice required a median of 4.1 hours to cool patients ($p < 0.1$). There was a trend for more patients to have favorable outcomes (CPC 1 or 2 at 30 days) after treatment with the faster cooling method, but the difference in outcomes did not reach statistical significance in this small study (46% vs. 38%, $p = .6$).

Castren et al [41] conducted a European multi-center trial of approximately 200 post-cardiac arrest patients in which the use of an ambulance-deployable nasal cooling device was compared with conventional hospital-based cooling techniques. The nasal cooling device enabled patients to be cooled to target core temperature within 2.1 hours of resuscitation, while in-hospital cooling enabled target core temperature to be reached 4.1 hours after resuscitation. In patients who received CPR within 10 minutes of arrest, the faster cooling approach yielded a 45.4% rate of neurologically intact survival to discharge (CPC 1-2 at discharge), while the slower approach yielded a 17.6% recovery rate ($p=.025$).

Peberdy et al [43] studied outcomes of 115 post-cardiac arrest patients treated in Richmond, VA over sequential time periods during which different cooling strategies were employed. During the first period of time, patients were only cooled in-hospital with surface cooling methods, reaching target temperature 6.6 hours after 911 call and achieving a 24% rate of neurologically intact survival to discharge. During the second period of time, 70% of patients received cooling in the field with cold IV saline, followed by treatment with cooling catheters within the hospital, reaching target temperature in an average of 4.1 hours after 911 call. This group achieved a 32% neurologically intact survival to discharge. The authors concluded that early initiation of hypothermia and rapid time to target temperature contributed to a strong trend for improved survival and neurologic outcomes for out-of-hospital cardiac arrest patients with VF.

The use of cold IV saline in pre-hospital patients has been used in a number of studies to achieve earlier initiation of therapeutic hypothermia [eg. 21, 22, 23, 43]. Despite encouraging results reported in some of these studies, this approach is limited in its cooling capability, and patients tend to passively rewarm following its discontinuation [22]. Stephen Bernard [44] reported the results of a 234-patient study which sought to demonstrate a benefit of field use of cold IV saline. Patients were randomized to receive cold IV saline in the field followed by surface cooling in the hospital, or to only receive surface cooling in the hospital. The study was terminated prematurely for futility, as it was observed that there was a trend for worsened outcomes in the patients treated with IV saline (49% survival) compared to those only receiving surface cooling in the hospital (51% survival). It is possible that the increase in cardiac pre-load which this method creates is poorly tolerated in some patients. Perhaps this or other adverse side effects of this technique negated the expected benefit of earlier cooling.

A study of 986 patients by Nielsen et al was unable to demonstrate a benefit of earlier cooling when results were subjected to analysis. However, 80% of the patients treated in this study received cold IV saline, which may have masked the benefits of earlier cooling. Furthermore, the patients in this study achieved target temperature an average of 5.8 hours after resuscitation. This would have missed the window for augmented release of the neuroprotective agent NPD-1, which in an earlier study [17] was observed to occur approximately 3 hours after resuscitation.

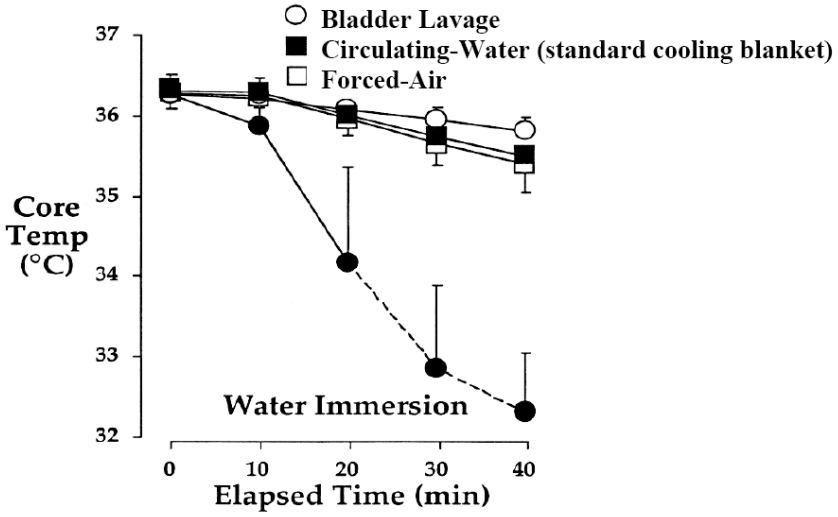
The above clinical data and previously cited animal data support the hypothesis that rapid early cooling may improve outcomes in post-arrest patients, compared to slower, later cooling. The

benefits of achieving rapid induction of therapeutic hypothermia are gaining increasing recognition. Polderman's review of therapeutic hypothermia [37] noted, "rapid induction decreases the risks and consequences of short-term side effects, such as shivering and metabolic disorders." The American Heart Association recently issued a policy [46] that calls for the establishment of level 1 and level 2 cardiac resuscitation centers for treatment of victims of cardiac arrest. An "essential element" of each such center is that it "initiates hypothermia as soon as feasible when indicated".

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 Cº/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] , bleeding [48] 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 rates of 0.65 to 1.2 Cº/hr with the Medivance Arctic Sun device [26, 42]).

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 34ºC in approximately 20 minutes (approximately 6.6 Cº/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 Cº/hr cooling rate).

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



A multicenter clinical study of the LRS ThermoSuit device [29] reported cooling rates similar to those of the above ice water immersion studies. In the initial series of patients treated, a median cooling time of only 37 minutes was required to induce hypothermia (cooling rate of 3.0 Cº/hr). The addition of a mild vasodilator (propofol) was found to reduce median cooling time to 27 minutes (cooling rate of 4.2 Cº/hr). The results of this study, when viewed in comparison with

those of a similar study of slower cooling methods, add further support for a benefit of more rapid achievement of therapeutic hypothermia (see Table 4 below):

Table 4: Comparison of Cooling Device Studies

Device:	**Cooling Blankets & Ice** Heard et al 2010	**Medivance Arctic Sun** Heard et al 2010	**LRS ThermoSuit** Howes et al 2010
Mean Age	64	57	64
% VF/VT	63%	68%	64%
% of patients reaching 34°C within 4 hours of start of cooling	50%	71%	100%
Median Cooling Time (inter-quartile range)	244 minutes (180-300)	190 minutes (135-155)	37 minutes (27-49)
% of Patients Surviving w. Good Outcomes (CPC 1,2)	**38%**	**47%**	**59%**

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 evidence in support of the need for cooling to be provided early for it to be most effective. Future clinical studies need to be conducted to assess the potential benefits of rapid cooling therapy for these and other new indications.

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.**

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