

Report Title

Final Progress Report for W911NF-05-1-0432, Hibernation Strategies to Improve Recovery from Hemorrhagic Shock

ABSTRACT

The ultimate goal of this project is to protect the warfighter from pathology that occurs as a result of significant blood loss. The overall strategy is to develop an effective fast-acting hemorrhagic shock protection fluid based on the molecular mechanisms used by hibernating mammals to survive reduced blood flow and avoid the consequences of ischemia and reperfusion injury.

The primary deliverable derived from this Phase 1 project is the ability to protect a non-hibernating mammal against injury from hemorrhagic shock. We have already shown in preliminary experiments that ischemic rat livers are protected from damage in vivo by administration of a preconditioning solution based on a molecular profile seen in hibernators. Optimization of a hemorrhagic shock protection fluid in non-hibernating rats, and assaying for their ability to protect against hemorrhagic shock, will serve as a prelude to Phase 2 of the Surviving Blood Loss Program.

The ultimate goal of our work is to protect the warfighter from pathology that occurs as a result of significant blood loss. This effort will concentrate on the preconditioning protection of two organs that are critical for successful recovery from hemorrhagic shock, the heart and brain.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Number of Papers published in peer-reviewed journals: 0.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Klein, A., Drewes, L.R., and Andrews, M.T. (2006) Hibernation Strategies to Improve Recovery from Hemorrhagic Shock. APS Conference on Comparative Physiology, Virginia Beach, VA, October 8-11, 2006, The Physiologist 49, C1-47, Abstract #27.1.

Number of Presentations: 1.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

Number of Manuscripts:

Number of Inventions:

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	
Amanda Klein	0.50	No
FTE Equivalent:	0.50	
Total Number:	1	

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	
FTE Equivalent:		
Total Number:		

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Matthew T. Andrews	0.25	No
Lester R. Drewes	0.10	No
FTE Equivalent:	0.35	
Total Number:	2	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	
Scott Wendroth	0.25	No
FTE Equivalent:	0.25	
Total Number:	1	

Names of Personnel receiving masters degrees

<u>NAME</u>	
Total Number:	

Names of personnel receiving PHDs

<u>NAME</u>	
Total Number:	

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	
FTE Equivalent:		
Total Number:		

Sub Contractors (DD882)

Inventions (DD882)

ARO Final Report
W911NF-05-1-0432
January 2007

Matthew T. Andrews, P.I.

Lester R. Drewes, Co-P.I.

Accomplishments Since Last Report

- Acute Blood Loss Experiments using D-BHB
 - Physiological monitoring completed
 - MABP, T_b , and Heart Rate (BPM)
 - New data from animals completed
- Survival Following Blood Return using D-BHB
 - Physiological monitoring completed
 - New data from animals completed

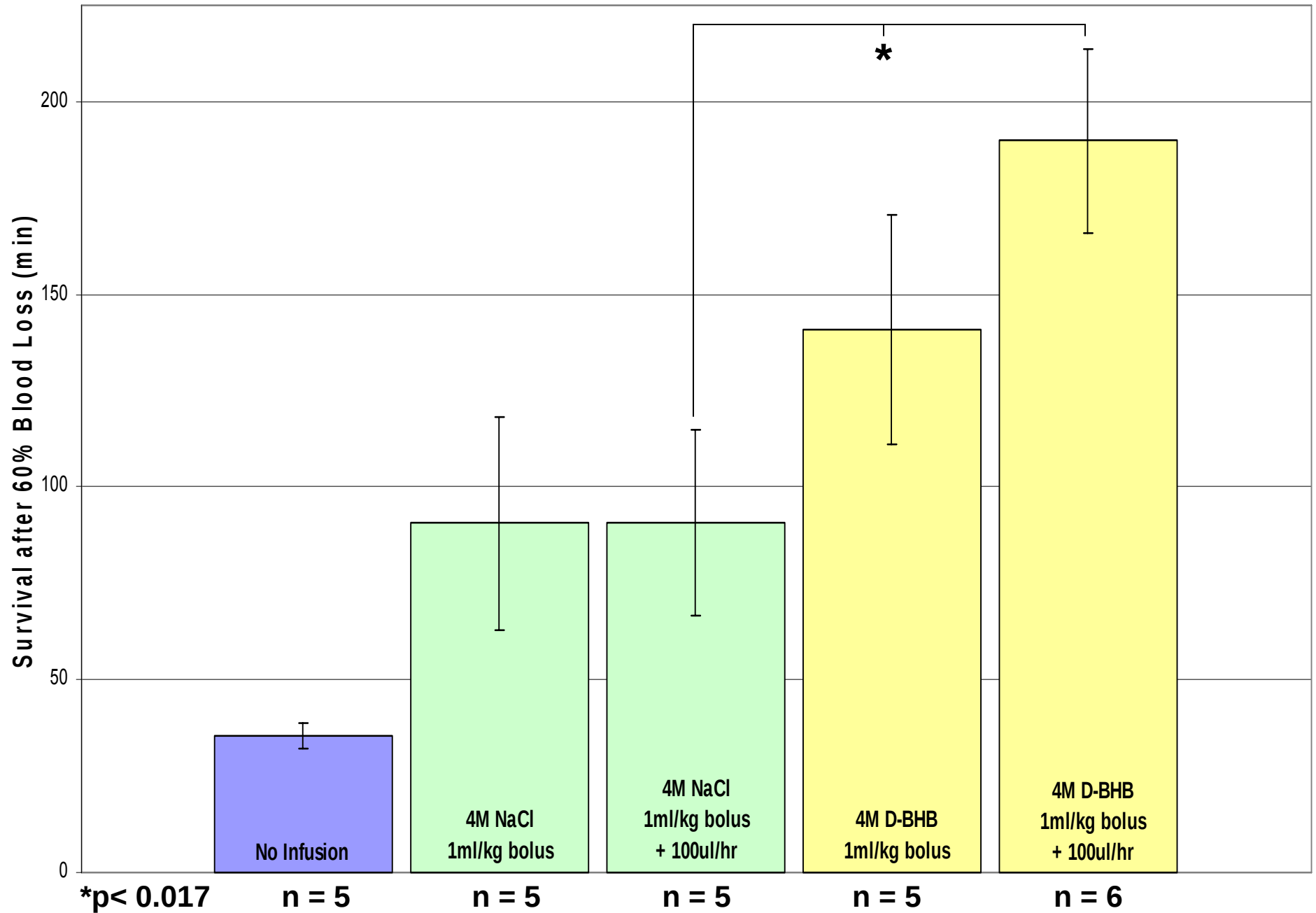
Acute Experiments

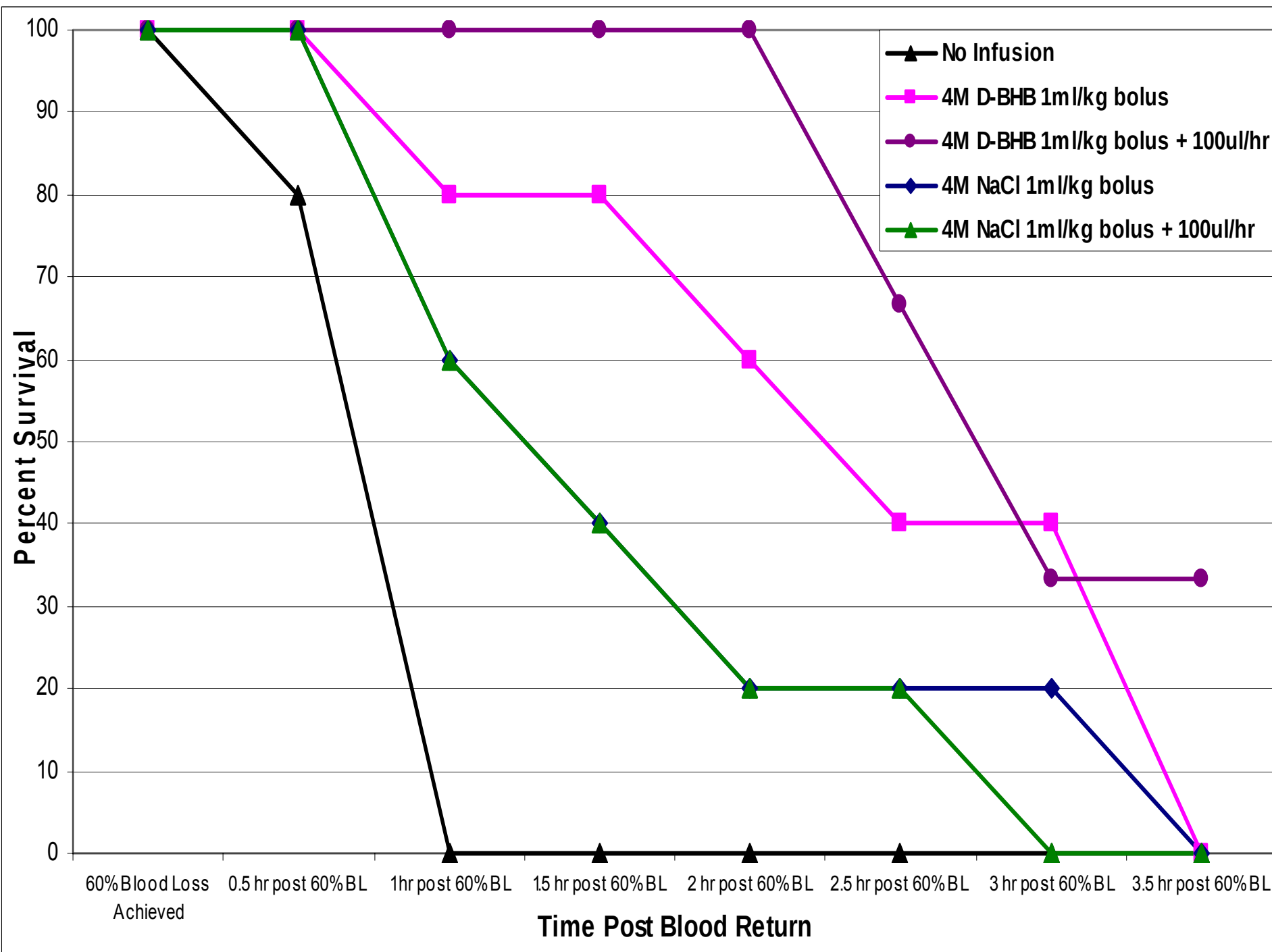
- Revised data on rats subjected to 60% blood loss
- Body temperatures allowed to cool to 27-29°C
- Therapies:
 - Control (no infusion)
 - 4M NaCl 1ml/kg bolus
 - 4M NaCl 1ml/kg bolus + 100 µl/hr infusion
 - 4M D-BHB 1ml/kg bolus
 - 4M D-BHB 1ml/kg bolus + 100 µl/hr infusion

Acute Experiments

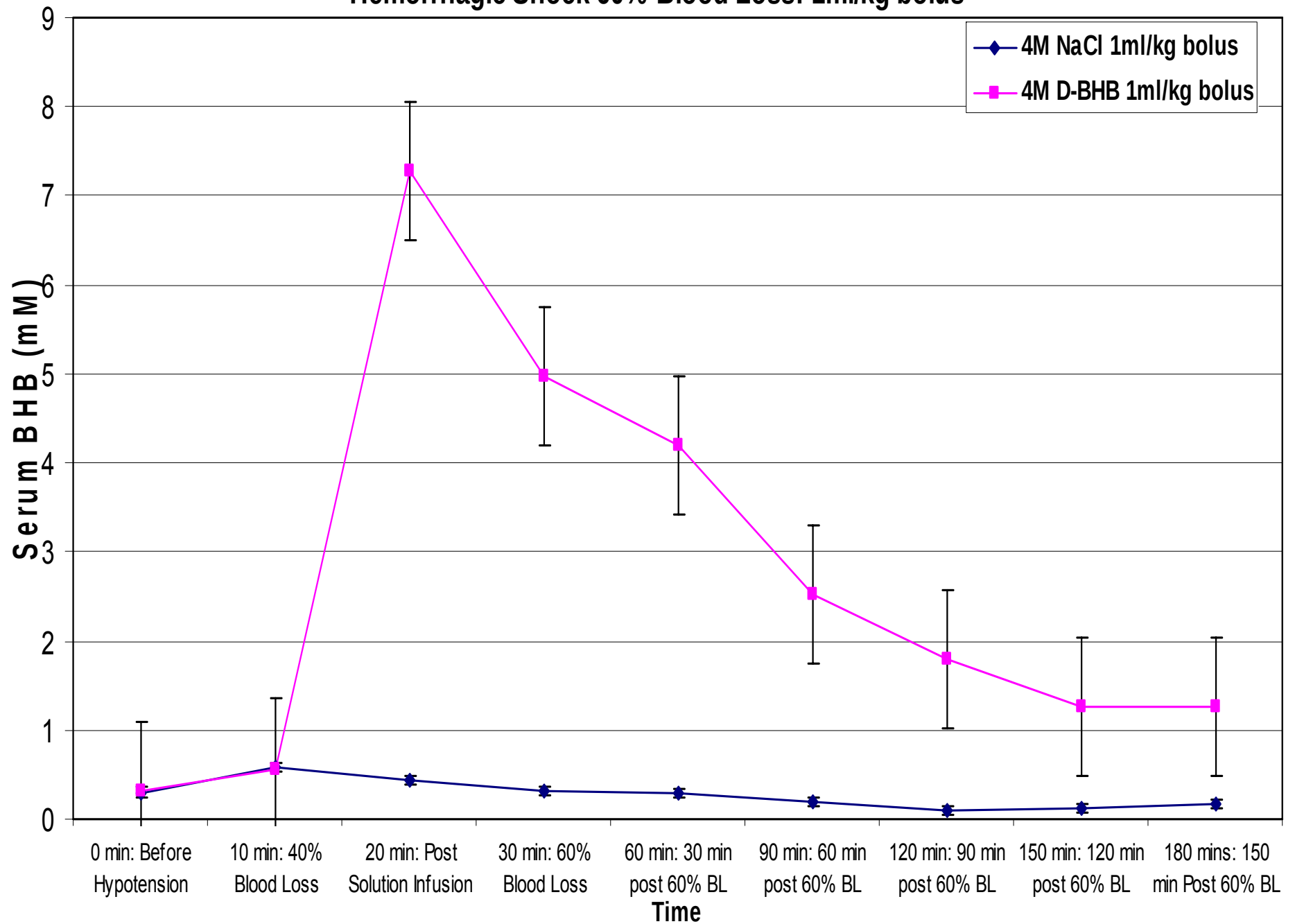
- 60% blood loss for 3 hours for a typical 300 g rat:
 - 10.8 ml blood removal
 - 600 μ l solution replaced (~3.3% of original blood volume)

Hemorrhagic Shock Model 60% Blood Loss: Acute Experiments

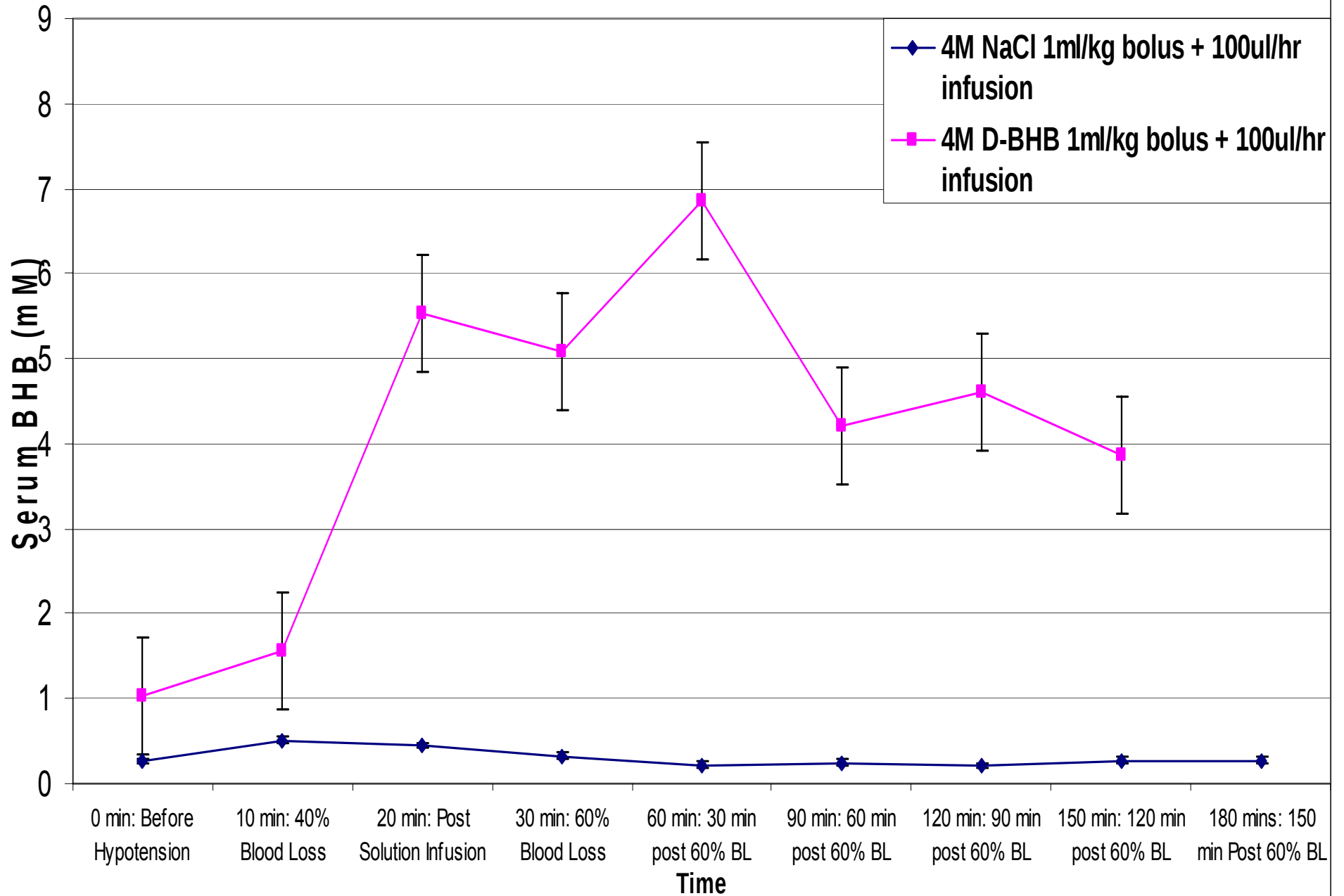




Hemorrhagic Shock 60% Blood Loss: 1ml/kg bolus



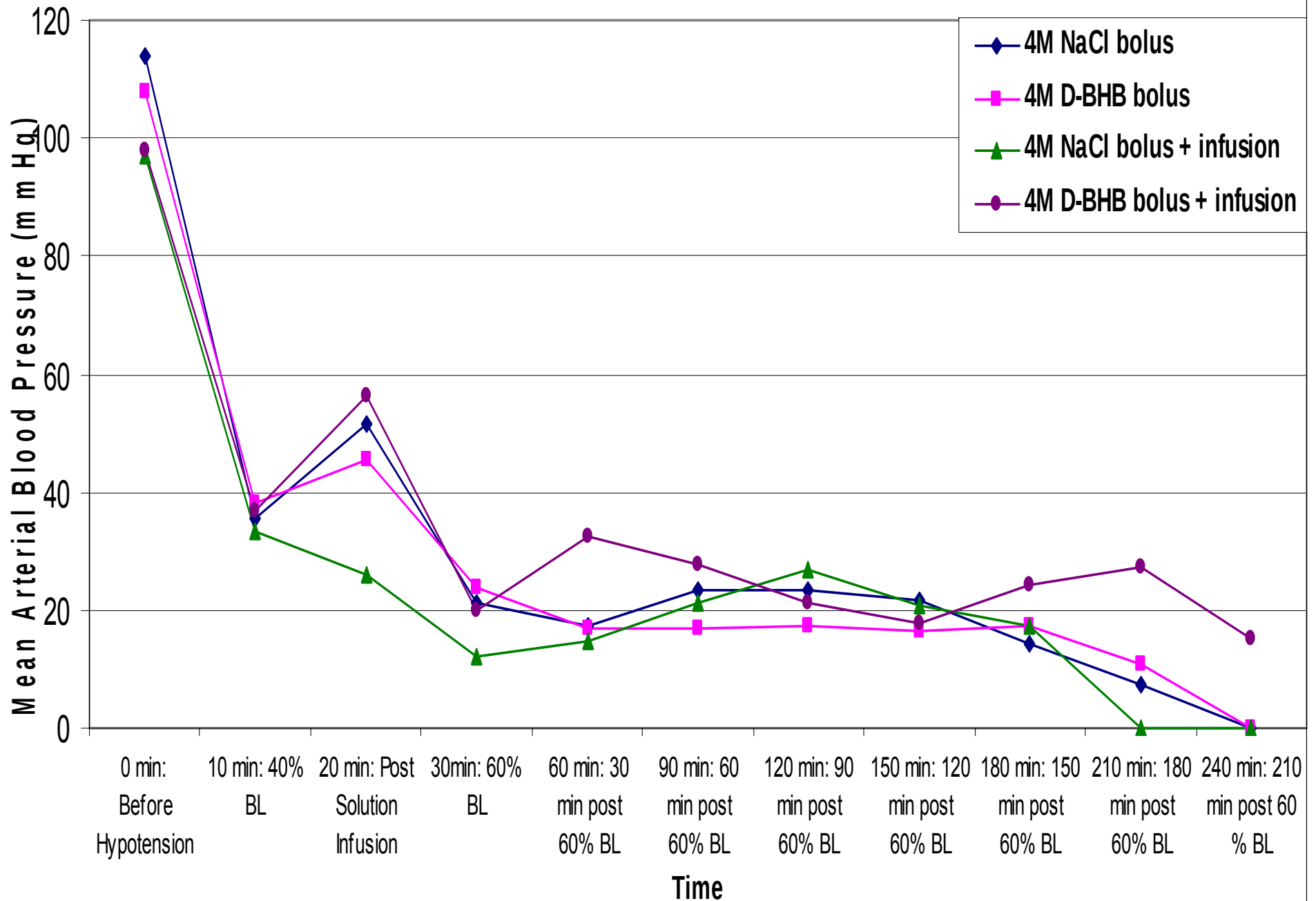
Hemorrhagic Shock 60% Blood Loss: 1ml/kg bolus + 100ul/hr infusion



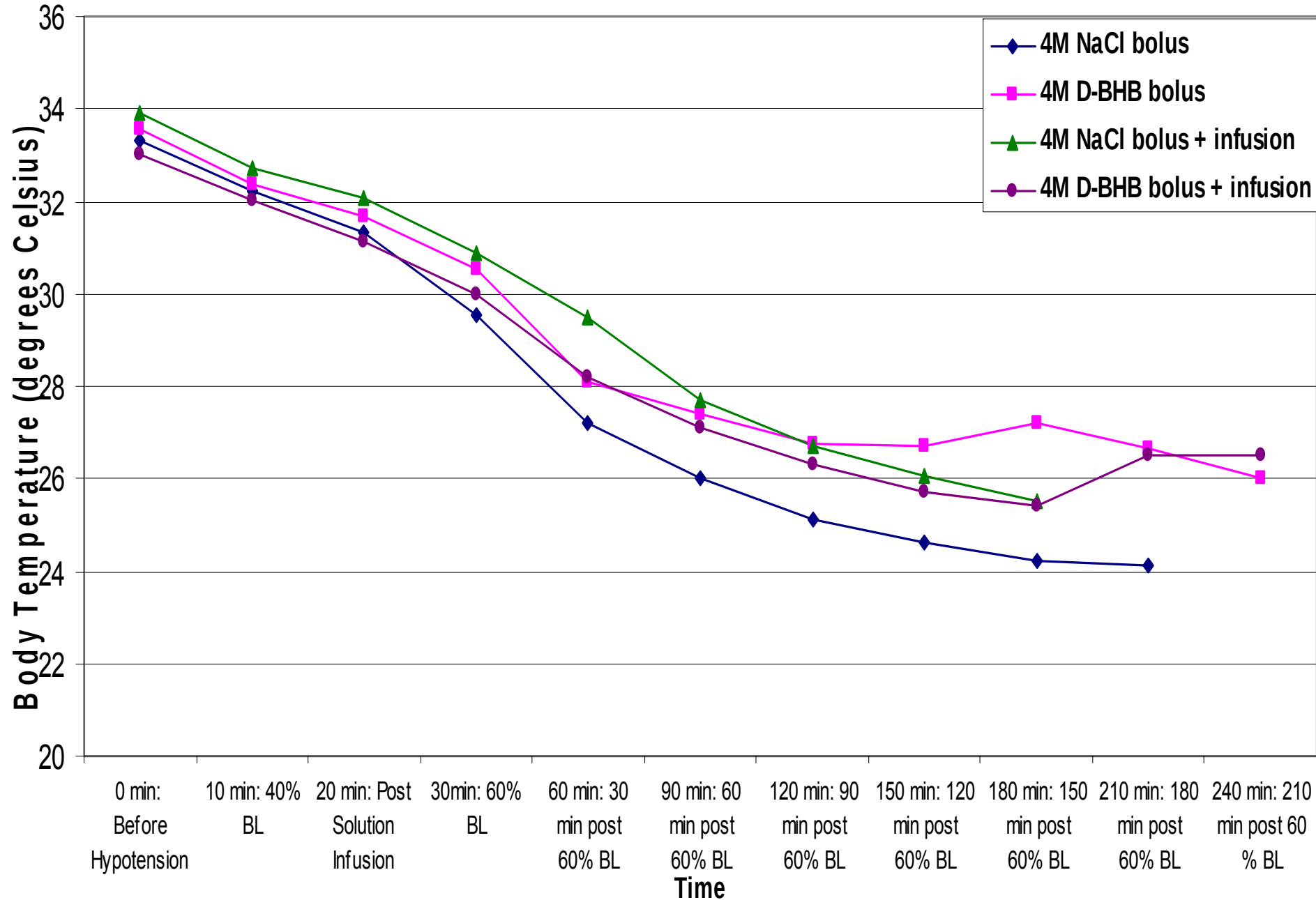
Physiological Monitoring: Acute Experiments

- What physiological parameters are improved upon infusion with D-BHB?
 - Mean Arterial Blood Pressure (MABP)
 - Body Temperature (T_b)
 - Heart Rate (BPM)
- Are these different from NaCl control?

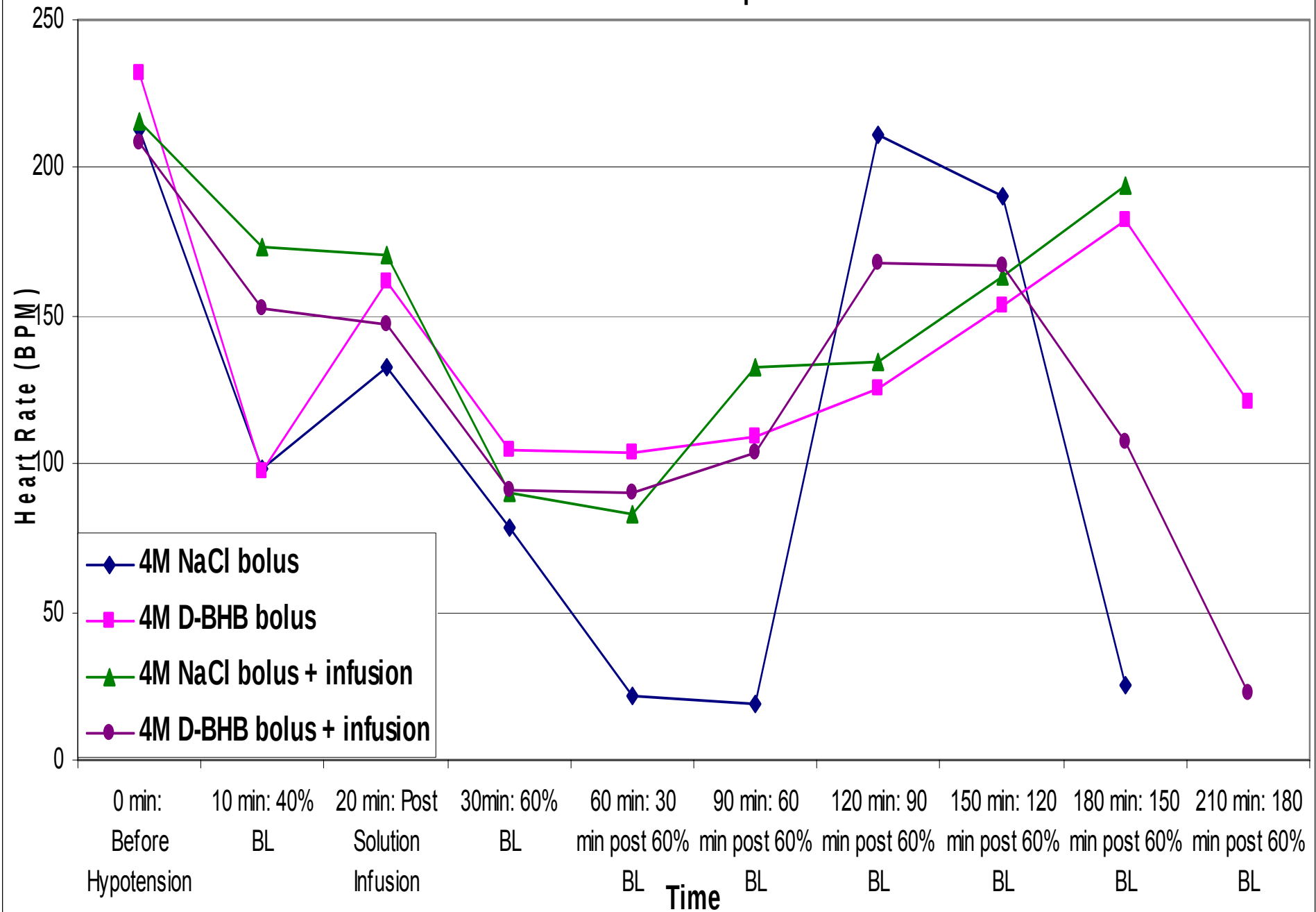
60% Blood Loss: Acute Experiments



60% Blood Loss: Acute Experiments



60% Blood Loss: Acute Experiments



Acute Experiment Conclusions

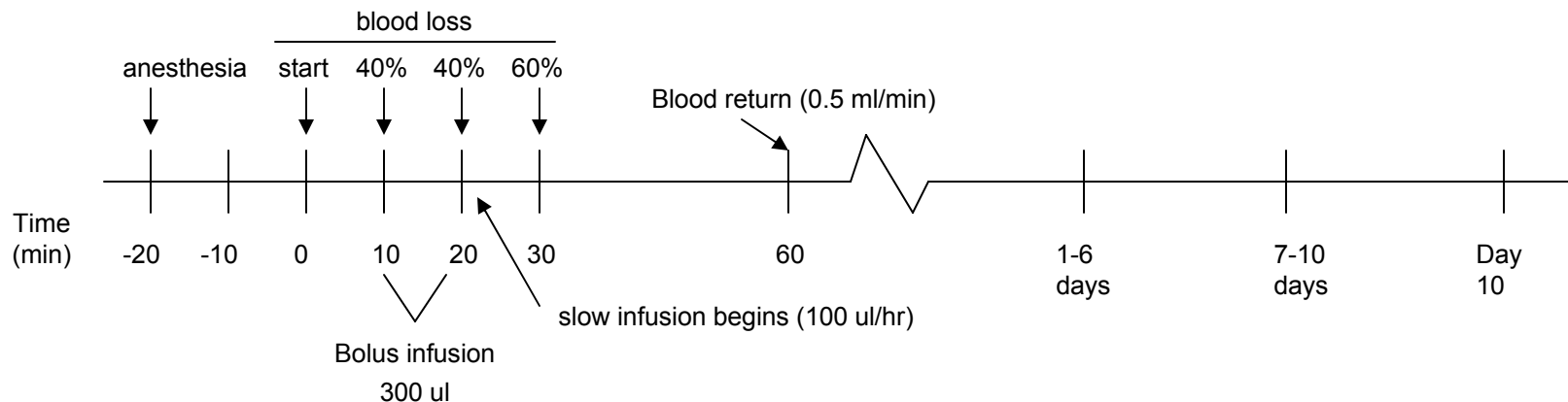
- Serum levels of BHB increase upon infusion
- Mean concentration of circulating BHB as high as 7 mM
- Maintaining elevated concentration of BHB prolongs survival of 60% blood loss to approx. 3hrs (mean = 189 min)
- Physiological parameters of MABP, T_b , and Heart Rate are generally the same with ketone and control solutions

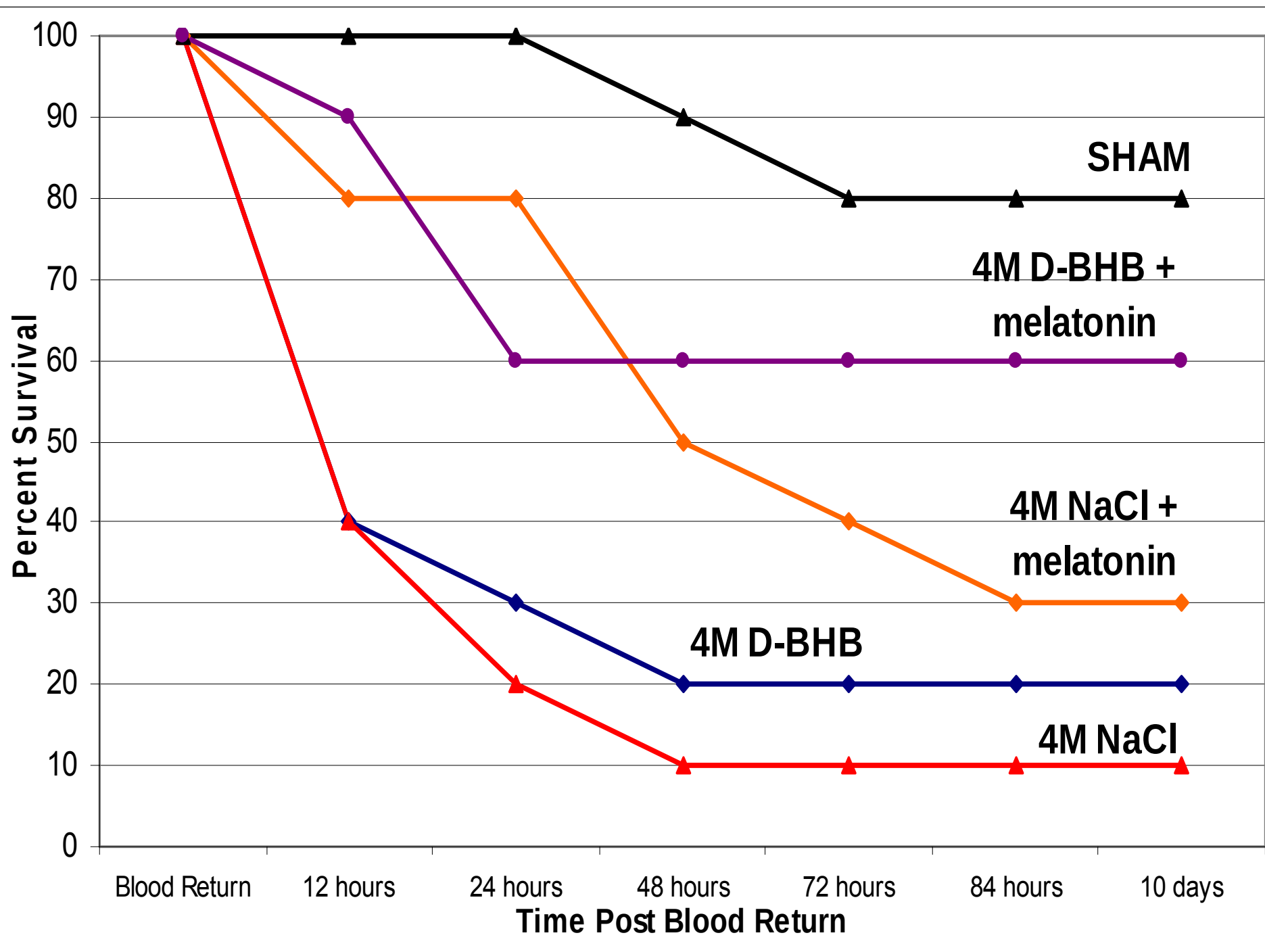
Survival Experiments

- Animals given shed blood after one hour post 60% blood loss achieved
- Blood return at 0.5 ml/min
- Temperature of shed blood is at the animal's body temperature (27°C – 29°C) when returned
- Animals allowed to recover
- SHAM animals: No blood loss, received anesthesia; and blood samples taken at equivalent time points

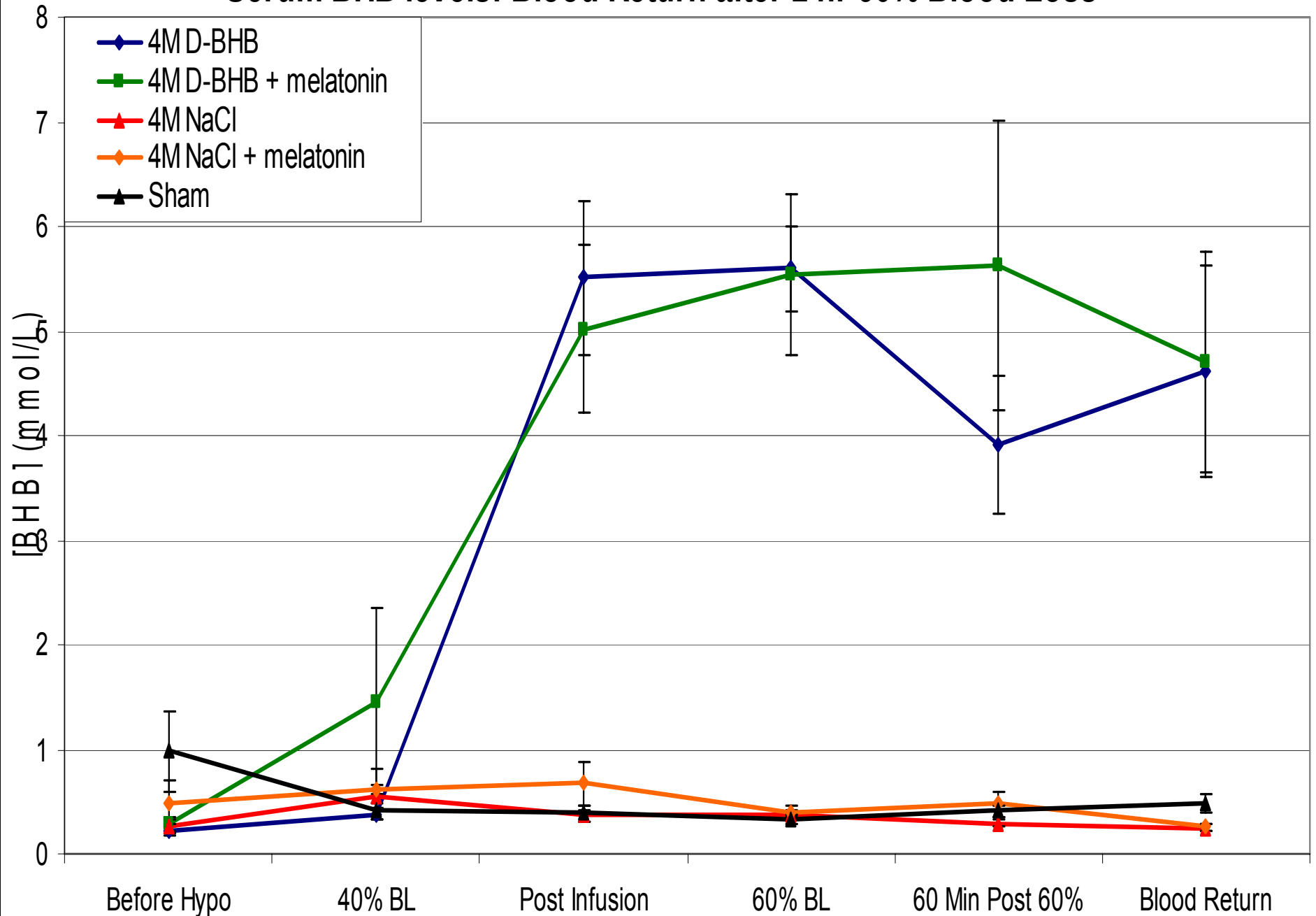
Blood Return Experiments

- Return shed blood after 1 hr at 60% BL
- Monitor rats quality of life post blood return
 - Day 1-6: Neurological Scoring
 - Day 7-10: Memory Testing
 - Day 10: Sacrifice → Brain Histology



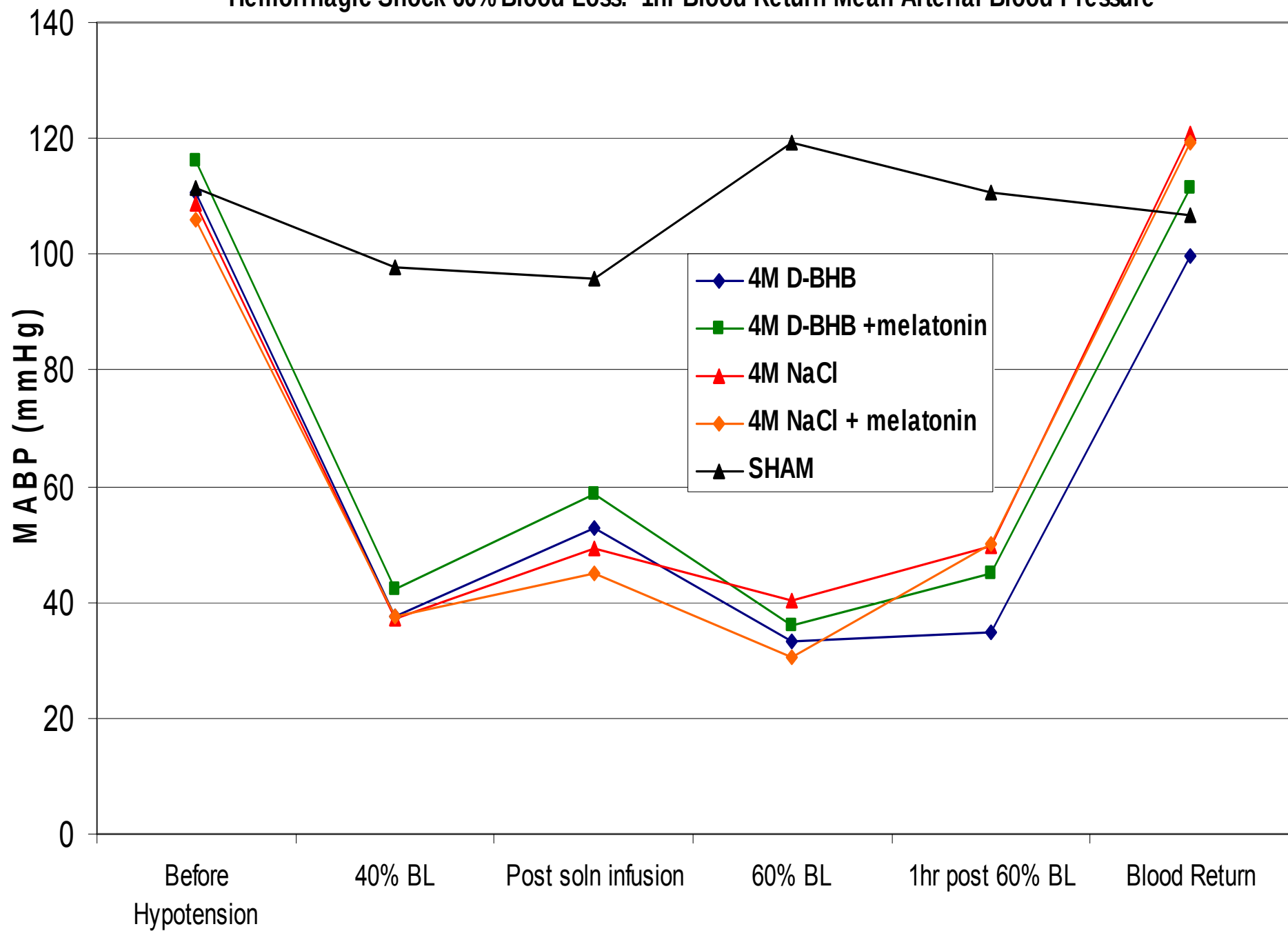


Serum BHB levels: Blood Return after 1 hr 60% Blood Loss

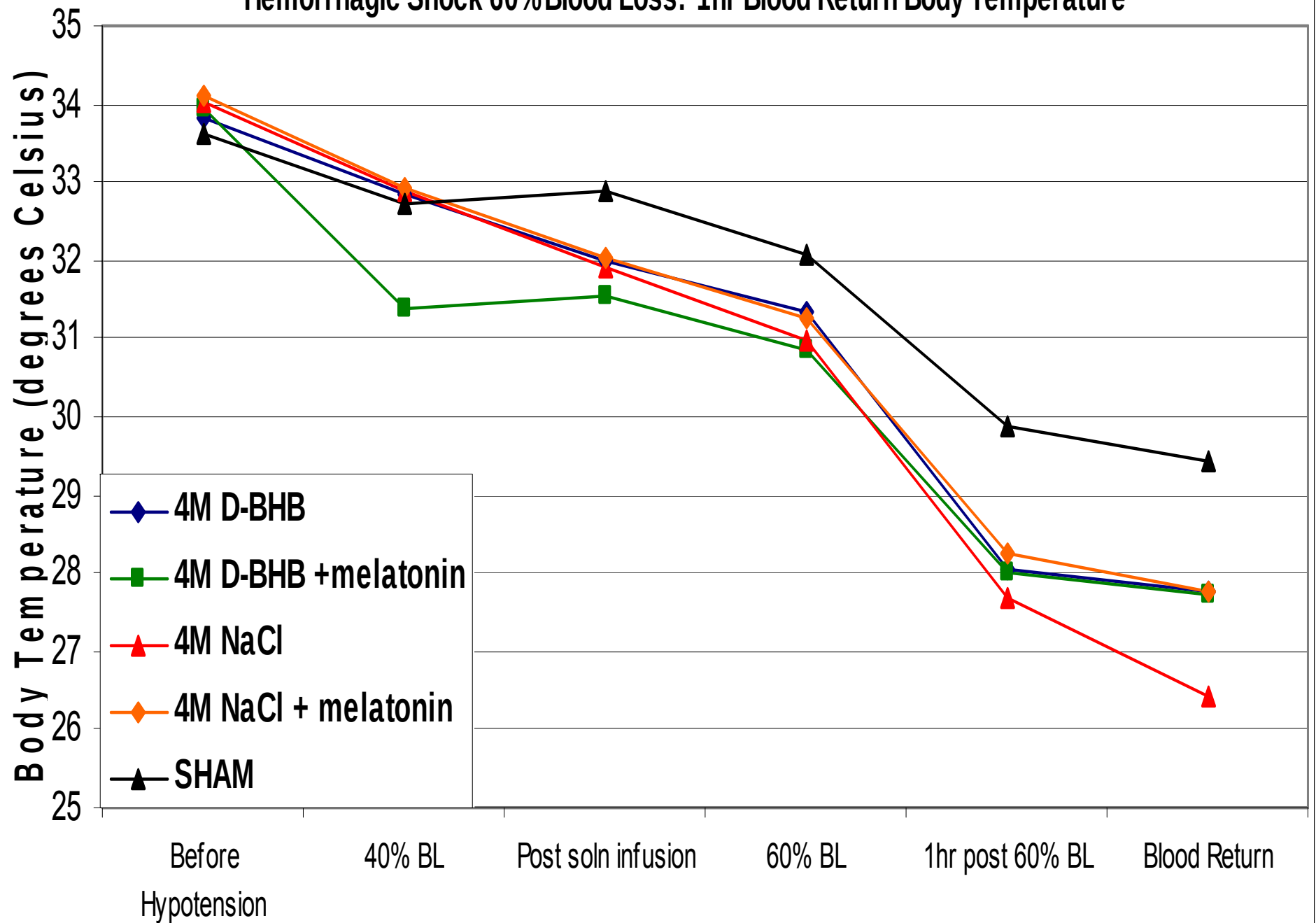


- Does infusion of D-BHB improve physiological parameters upon 60% blood loss?
 - Mean Arterial Blood Pressure (MABP)
 - Body Temperature (T_b)
 - Heart Rate (BPM)
- Are these different from our control, NaCl?

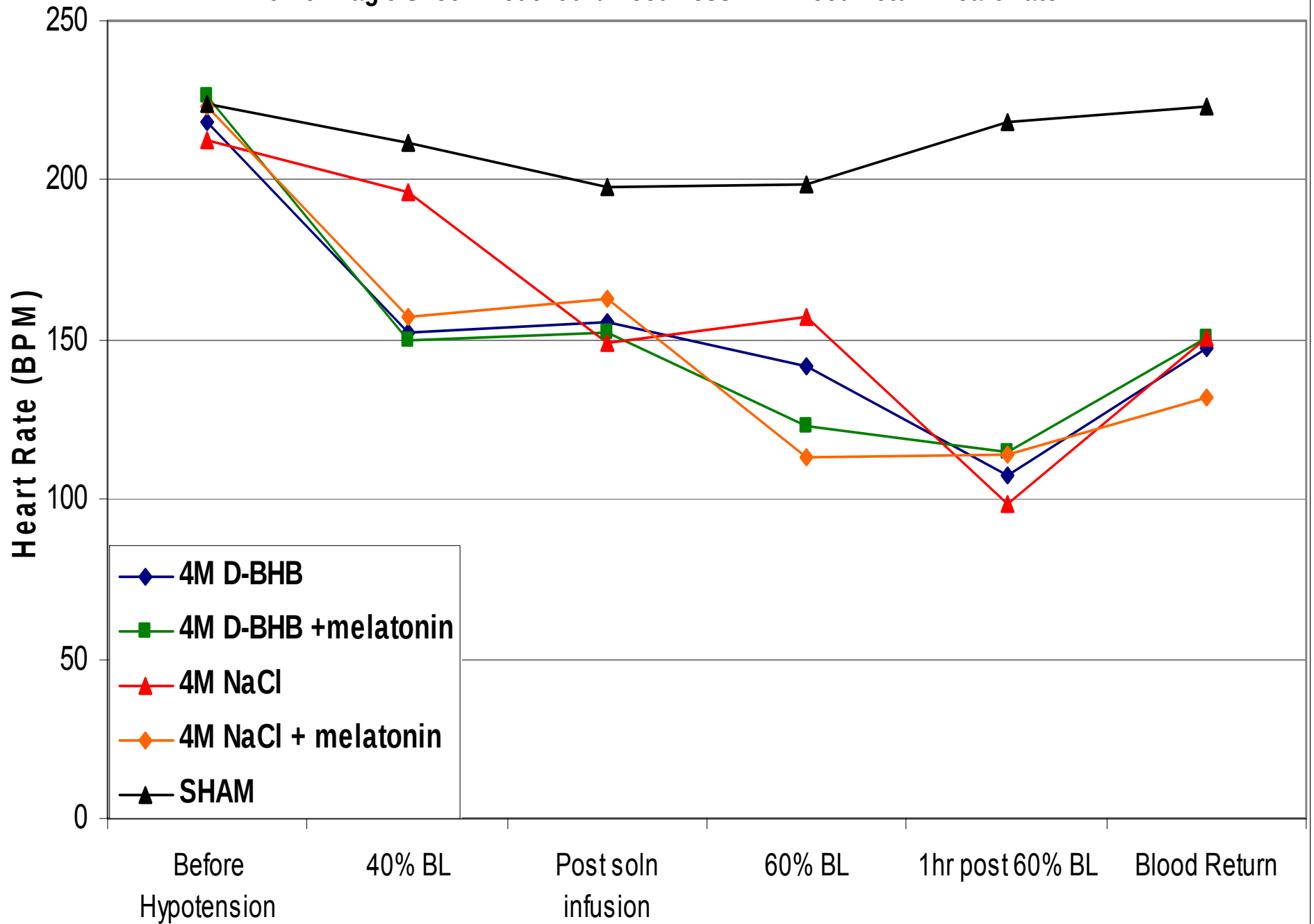
Hemorrhagic Shock 60% Blood Loss: 1hr Blood Return Mean Arterial Blood Pressure



Hemorrhagic Shock 60% Blood Loss: 1hr Blood Return Body Temperature



Hemorrhagic Shock Model 60% Blood Loss: 1hr Blood Return Heart Rate



Survival Experimental Conclusions

- Kaplan-Meier graph shows 4M D-BHB + melatonin as greatest survival compared to SHAM animals (n=10)
- Addition of the antioxidant melatonin may play big role in aiding against reperfusion injury upon blood return
- Physiological parameters of MABP, T_b , and Heart Rate are generally the same with ketone and control infusions

Ongoing Experiments

- Survival Experiments: Data collection and analyze results
 - Neurological Scoring (Days 1-6)
 - Basic behaviors
 - Memory Testing (Days 7-10)
 - Higher order brain processing
 - Histology (Day 10)
 - Brain damage due to 60% blood loss for one hour